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NEWS	6	MAR 03	REGISTRY/ZREGISTRY - Sequence annotations enhanced
NEWS	7	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	8	MAR 22	KOREAPAT now updated monthly; patent information enhanced
NEWS	9	MAR 22	Original IDE display format returns to REGISTRY/ZREGISTRY
NEWS	10	MAR 22	PATDPASPC - New patent database available
NEWS	11	MAR 22	REGISTRY/ZREGISTRY enhanced with experimental property tags
NEWS	12	APR 04	EPFULL enhanced with additional patent information and new fields
NEWS	13	APR 04	EMBASE - Database reloaded and enhanced
NEWS	14	APR 18	New CAS Information Use Policies available online
NEWS	15	APR 25	Patent searching, including current-awareness alerts (SDIs), based on application date in CA/CAplus and USPATFULL/USPAT2 may be affected by a change in filing date for U.S. applications.
NEWS	16	APR 28	Improved searching of U.S. Patent Classifications for U.S. patent records in CA/CAplus
NEWS	17	MAY 23	GBFULL enhanced with patent drawing images
NEWS	18	MAY 23	REGISTRY has been enhanced with source information from CHEMCATS
NEWS	19	JUN 06	The Analysis Edition of STN Express with Discover! (Version 8.0 for Windows) now available
NEWS	20	JUN 13	RUSSIAPAT: New full-text patent database on STN
NEWS	21	JUN 13	FRFULL enhanced with patent drawing images
NEWS	22	JUN 27	MARPAT displays enhanced with expanded G-group definitions and text labels
NEWS	23	JUL 01	MEDICONF removed from STN
NEWS	24	JUL 07	STN Patent Forums to be held in July 2005
NEWS	25	JUL 13	SCISEARCH reloaded

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FILE 'LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005
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=> s "PDK1"
L1          1799 "PDK1"

=> s phosphoinositide##
L2         62622 PHOSPHOINOSITIDE##

=> s l1 and l2
L3          1051 L1 AND L2

=> s  "PIF" or "PRK2"
L4          2934 "PIF" OR "PRK2"

=> s l3 and l4
L5            78 L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
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=> s "serine 473"
L7          528 "SERINE 473"

=> s l6 and l7
L8            0 L6 AND L7

=> d l6 1-24 ibib ab

L6   ANSWER 1 OF 24  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2005:182830  HCAPLUS
DOCUMENT NUMBER:          142:275030
TITLE:                    Expression of 3-phosphoinositide-dependent
                          protein kinase 1 (PDK-1) inhibitor in mammalian cells
                          and uses in treating diseases related to
                          phosphorylation of PDK-1
INVENTOR(S):              Sakai, Norio
PATENT ASSIGNEE(S):       Japan Science and Technology Agency, Japan
SOURCE:                   PCT Int. Appl., 48 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 Japanese
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.          DATE
     ---------------      ----   --------    --------------------     --------
     WO 2005019451         A1    20050303    WO 2004-JP4536           20040330
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
             LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ,
             BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE,
             ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
             SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN,
             TD, TG
PRIORITY APPLN. INFO.:                        JP 2003-298760      A  20030822
AB   The invention relates to 3-phosphoinositide-dependent protein
     kinase 1 (PDK-1) inhibitors and uses in treating diseases related to
     phosphorylation of PDK-1. The sequences of inhibitors for 3-
     phosphoinositide-dependent protein kinase 1 (PDK-1) are provided.
     PDK-1-interacting fragment (PIF) is fused with activation loop
     of protein kinase B and green fluorescent protein to construct vector
     PKBAL-PIF-GFP, or further fused with signal peptide from
     tyrosine phosphatase Lyn to construct vector Lynsig-PKBAL-PIF
     -GFP.  PDK-1-interacting fragment (PIF) is also fused with
     activation loop of protein kinase C, δ and green fluorescent protein
     to construct vector δAL- PIF-GFP, or further fused with
     signal peptide from tyrosine phosphatase Lyn to construct vector
     Lynsig-δAL- PIF-GFP.  The δAL- PIF-GFP and
     Lynsig-δAL- PIF-GFP vectors were injected into the
     tumor-derived cell lines COS-7 and PC-12 to induce apoptosis, chromatin
     condensation and nucleus fragmentation.
REFERENCE COUNT:          9     THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6   ANSWER 2 OF 24     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2004501625     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 15470109
TITLE:               Differential roles of PDK1- and
                     PDK2-phosphorylation sites in the yeast AGC kinases Ypk1,
```

Pkc1 and Sch9.
AUTHOR: Roelants Francoise M; Torrance Pamela D; Thorner Jeremy
CORPORATE SOURCE: Department of Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720-3202, USA.
CONTRACT NUMBER: CA09041 (NCI)
GM07232 (NIGMS)
GM21841 (NIGMS)
SOURCE: Microbiology (Reading, England), (2004 Oct) 150 (Pt 10) 3289-304.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200501
ENTRY DATE: Entered STN: 20041008
Last Updated on STN: 20050114
Entered Medline: 20050113

AB Saccharomyces cerevisiae Pkh1 and Pkh2 (orthologues of mammalian protein kinase, **PDK1**) are functionally redundant. These kinases activate three AGC family kinases involved in the maintenance of cell wall integrity: Ypk1 and Ypk2, two closely related, functionally redundant enzymes (orthologues of mammalian protein kinase SGK), and Pkc1 (orthologue of mammalian protein kinase **PRK2**). Pkh1 and Pkh2 activate Ypk1, Ypk2 and Pkc1 by phosphorylating a Thr in a conserved sequence motif (**PDK1** site) within the activation loop of these proteins. A fourth protein kinase involved in growth control and stress response, Sch9 (orthologue of mammalian protein kinase c-Akt/PKB), also carries the conserved activation loop motif. Like other AGC family kinases, Ypk1, Ypk2, Pkc1 and Sch9 also carry a second conserved sequence motif situated in a region C-terminal to the catalytic domain, called the hydrophobic motif (PDK2 site). Currently, there is still controversy surrounding the identity of the enzyme responsible for phosphorylating this second site and the necessity for phosphorylation at this site for in vivo function. Here, genetic and biochemical methods have been used to investigate the physiological consequences of phosphorylation at the **PDK1** and PDK2 sites of Ypk1, Pkc1 and Sch9. It was found that phosphorylation at the **PDK1** site in the activation loop is indispensable for the essential functions of all three kinases in vivo, whereas phosphorylation at the PDK2 motif plays a non-essential and much more subtle role in modulating the ability of these kinases to regulate the downstream processes in which they participate.

L6 ANSWER 3 OF 24 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2004251330 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15116068
TITLE: The in vivo role of PtdIns(3,4,5)P3 binding to **PDK1** PH domain defined by knockin mutation.
AUTHOR: McManus Edward J; Collins Barry J; Ashby Peter R; Prescott Alan R; Murray-Tait Victoria; Armit Laura J; Arthur J Simon C; Alessi Dario R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, School of Life Sciences, MSI/WTB complex, University of Dundee, Dundee, UK.. e.j.mcmanus@dundee.ac.uk
SOURCE: EMBO journal, (2004 May 19) 23 (10) 2071-82. Electronic Publication: 2004-04-29.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20040520

Last Updated on STN: 20041219

AB We generated homozygous knockin ES cells expressing a form of 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) with a mutation in its pleckstrin homology (PH) domain that abolishes phosphatidylinositol 3,4,5-tris-phosphate (PtdIns(3,4,5)P3) binding, without affecting catalytic activity. In the knockin cells, protein kinase B (PKB) was not activated by IGF1, whereas ribosomal S6 kinase (RSK) was activated normally, indicating that PtdIns(3,4,5)P3 binding to **PDK1** is required for PKB but not RSK activation. Interestingly, amino acids and Rheb, but not IGF1, activated S6K in the knockin cells, supporting the idea that PtdIns(3,4,5)P3 stimulates S6K through PKB-mediated activation of Rheb. Employing **PDK1** knockin cells in which either the PtdIns(3,4,5)P3 binding or substrate-docking ' **PIF** pocket' was disrupted, we established the roles that these domains play in regulating phosphorylation and stabilisation of protein kinase C isoforms. Moreover, mouse **PDK1** knockin embryos in which either the PH domain or **PIF** pocket was disrupted died displaying differing phenotypes between E10.5 and E11.5. Although **PDK1** plays roles in regulating cell size, cells derived from PH domain or **PIF** pocket knockin embryos were of normal size. These experiments establish the roles of the **PDK1** regulatory domains and illustrate the power of knockin technology to probe the physiological function of protein-lipid and protein-protein interactions.

L6 ANSWER 4 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:109910 HCAPLUS
DOCUMENT NUMBER: 140:353654
TITLE: A protein kinase target of a **PDK1** signalling pathway is involved in root hair growth in Arabidopsis
AUTHOR(S): Anthony, Richard G.; Henriques, Rossana; Helfer, Anne; Meszaros, Tamas; Rios, Gabino; Testerink, Christa; Munnik, Teun; Deak, Maria; Koncz, Csaba; Boegre, Laszlo
CORPORATE SOURCE: School of Biological Sciences, University of London, Surrey, UK
SOURCE: EMBO Journal (2004), 23(3), 572-581
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Here we report on a lipid-signalling pathway in plants that is downstream of phosphatidic acid and involves the Arabidopsis protein kinase, AGC2-1, regulated by the 3'-**phosphoinositide**-dependent kinase-1 (AtPDK1). AGC2-1 specifically interacts with AtPDK1 through a conserved C-terminal hydrophobic motif that leads to its phosphorylation and activation, whereas inhibition of AtPDK1 expression by RNA interference abolishes AGC2-1 activity. Phosphatidic acid specifically binds to AtPDK1 and stimulates AGC2-1 in an AtPDK1-dependent manner. AtPDK1 is ubiquitously expressed in all plant tissues, whereas expression of AGC2-1 is abundant in fast-growing organs and dividing cells, and activated during re-entry of cells into the cell cycle after sugar starvation-induced G1-phase arrest. Plant hormones, auxin and cytokinin, synergistically activate the AtPDK1-regulated AGC2-1 kinase, indicative of a role in growth and cell division. Cellular localization of GFP-AGC2-1 fusion protein is highly dynamic in root hairs and at some stages confined to root hair tips and to nuclei. The agc2-1 knockout mutation results in a reduction of root hair length, suggesting a role for AGC2-1 in root hair growth and development.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:114175 BIOSIS

DOCUMENT NUMBER: PREV200500111591
TITLE: Functional analysis of **PDK1** signalling pathway using knockout and knockin approaches.
AUTHOR(S): McManus, Ed J.; Collins, Barry J.; Mora, Alfonso; Alessi, Dario R.
SOURCE: Biochemical Society Transactions, (August 2004) Vol. 32, No. Part 4, pp. 38A. print.
Meeting Info.: BioScience2004: From Molecules to Organisms. Glasgow, UK. July 18-22, 2004. The Biochemical Society.
CODEN: BCSTB5. ISSN: 0300-5127.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Mar 2005
Last Updated on STN: 23 Mar 2005

L6 ANSWER 6 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:991698 HCAPLUS
DOCUMENT NUMBER: 140:37976
TITLE: Crystal structures of human **phosphoinositide**-dependent protein kinase **PDK1** complexes and method for identifying modulators of **PDK1** activity
INVENTOR(S): Alessi, Dario; Biondi, Ricardo; Komander, David; Van Aalten, Daan
PATENT ASSIGNEE(S): University of Dundee, UK
SOURCE: PCT Int. Appl., 383 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003104481	A2	20031218	WO 2003-GB2509	20030609
WO 2003104481	A3	20040923		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1513947	A2	20050316	EP 2003-730356	20030609
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				

PRIORITY APPLN. INFO.: GB 2002-13186 A 20020608
WO 2003-GB2509 W 20030609

AB A method for selecting or designing a compound for modulating the activity of **phosphoinositide** dependent protein kinase 1 (PDKI) comprises using mol. modeling means to select or design a compound that is predicted to interact with the protein kinase catalytic domain of PDKI, wherein a 3D structure of at least a part of the protein kinase catalytic domain of **PDK1** is compared with a three-dimensional structure of a compound Thus, the crystal structure of residues 51 to 359 of human **PDK1** complexed with ATP was determined to 2Å and that of the catalytic domain complexed with staurosporine or with UCN-01 was determined to 2.3 and 2.5Å, resp. A phosphopeptide binding domain consisting of an hydrophobic pocket (**PIF** binding pocket) defined by residues including Lys115, 20

Ile118, Ile119, Val124, Val127 and/or Leu155 and a phosphate binding pocket defined by residues including Lys76, Arg131, Thr148 and/or Gln150 were identified by anal. of the crystal structure and by mutational anal. UCN-01 was found not to be a specific kinase inhibitor since it inhibited over half of a panel of 29 protein kinases.

L6 ANSWER 7 OF 24 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003377319 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12912918
TITLE: In vivo role of the **PIF**-binding docking site of **PDK1** defined by knock-in mutation.
AUTHOR: Collins Barry J; Deak Maria; Arthur J Simon C; Armit Laura J; Alessi Dario R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, UK.. b.j.collins@dundee.ac.uk
SOURCE: EMBO journal, (2003 Aug 15) 22 (16) 4202-11. Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200310
ENTRY DATE: Entered STN: 20030813
Last Updated on STN: 20031004
Entered Medline: 20031003

AB PKB/Akt, S6K, SGK and RSK are mediators of responses triggered by insulin and growth factors and are activated following phosphorylation by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**). To investigate the importance of a substrate-docking site in the kinase domain of **PDK1** termed the '**PIF**-pocket', we generated embryonic stem (ES) cells in which both copies of the **PDK1** gene were altered by knock-in mutation to express a form of **PDK1** retaining catalytic activity, in which the **PIF**-pocket site was disrupted. The knock-in ES cells were viable, mutant **PDK1** was expressed at normal levels and insulin-like growth factor 1 induced normal activation of PKB and phosphorylation of the PKB substrates GSK3 and FKHR. In contrast, S6K, RSK and SGK were not activated, nor were physiological substrates of S6K and RSK phosphorylated. These experiments establish the importance of the **PIF**-pocket in governing the activation of S6K, RSK, SGK, but not PKB, in vivo. They also illustrate the power of knock-in technology to probe the physiological roles of docking interactions in regulating the specificity of signal transduction pathways.

L6 ANSWER 8 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:317639 SCISEARCH
THE GENUINE ARTICLE: 664UR
TITLE: PKC epsilon is a permissive link in integrin-dependent IFN-gamma signalling that facilitates JAK phosphorylation of STAT1
AUTHOR: Ivaska J (Reprint); Bosca L; Parker P J
CORPORATE SOURCE: Canc Res UK London Res Inst, Prot Phosphorylat Lab, Lincolns Inn Fields Labs, 44 Lincolns Inn Fields, London WC2A 3PX, England (Reprint); Canc Res UK London Res Inst, Prot Phosphorylat Lab, Lincolns Inn Fields Labs, London WC2A 3PX, England; CSIC, Inst Bioquim, UCM, Fac Farm, E-28040 Madrid, Spain
COUNTRY OF AUTHOR: England; Spain
SOURCE: NATURE CELL BIOLOGY, (APR 2003) Vol. 5, No. 4, pp. 363-369

ISSN: 1465-7392.

PUBLISHER: NATURE PUBLISHING GROUP, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND.
DOCUMENT TYPE: Letter; Journal
LANGUAGE: English
REFERENCE COUNT: 25
ENTRY DATE: Entered STN: 25 Apr 2003
Last Updated on STN: 25 Apr 2003
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The critical dependence of receptor-triggered signals on integrin-mediated cell-substrate. interactions represents a fundamental biological paradigm in health and disease. However, the molecular connections of these permissive inputs, which operate through integrin-matrix interactions, has remained largely obscure. Here we show that the serine-threonine kinase protein kinase C epsilon (PKCepsilon) functions as a signal integrator between cytokine and integrin signalling pathways. Integrins are shown to control PKCepsilon phosphorylation acutely by determining complex formation with protein phosphatase 2A (PP2A) and the upstream kinase **PDK1** (**phosphoinositide** -dependent kinase 1). The PP2A-induced loss of PKCepsilon function results in attenuated interferon gamma (INF-gamma)-induced phosphorylation of STAT1 (signal transducer and activator of transcription 1) downstream of Janus kinase 1/2 (JAK1/2). PKCepsilon function and the IFN-gamma response can be recovered by inhibition of PP2A if **PDK1** is associated with PKCepsilon in this complex. More directly, a PP2A-resistant mutant of PKCepsilon is sufficient for restoration of the IFN-gamma response in suspension culture. Thus, PKCepsilon functions as a central point of integration through which integrin engagement exerts a permissive input on IFN-gamma signalling.

L6 ANSWER 9 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:204833 BIOSIS
DOCUMENT NUMBER: PREV200400205373
TITLE: The effect of Akt by antidepressants in the rat brain.
AUTHOR(S): Misonoo, A. [Reprint Author]; Kenichi, O. [Reprint Author]; Hsagawa, H. [Reprint Author]; Kiyofumi, T. [Reprint Author]; Kanai, S. [Reprint Author]; Tanaka, D. [Reprint Author]; Hisinuma, T. [Reprint Author]; Fujii, S. [Reprint Author]; Sasuga, Y. [Reprint Author]; Miyamoto, S. [Reprint Author]; Asakura, M. [Reprint Author]
CORPORATE SOURCE: Dept. Neuropsych, St. Marianna Univ. Sch. Med, Kawasaki, Japan
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 849.15. http://sfn.scholarone.com. e-file.
Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Apr 2004
Last Updated on STN: 14 Apr 2004

AB Akt, also known as protein kinase B, is a protein kinase as a downstream kinase of **phosphoinositide** 3-kinase (PI3-K) and BDNF. Phoshporylation of residues Ser-473 and Thr-308 is required for Akt activity by **PDK1** and PDK2, respectively. **PRK2** inhibits the phosphorylation of Akt Ser-473 by **PDK1**. Key roles for Akt in cellular processes such as apotosis, neurotransmitters release and transcription are now well established.The phosphorylation of Akt Ser-473 and Thr-308 increased after 3 weeks Clomipramine and Fluvoxamine treatment by Immunoblot measurement. **PDK1** and **PDK1**, Ser-241 phosphorylation also increased after treatment of antidepressants. But PI3-K and **PRK2** were not changed by antidepressants.Akt is

known to play a role in the releasing process for several neurotransmitters (5-HT and NE). It is important cellular mechanism for antidepressants that Akt activated by PDK.

L6 ANSWER 10 OF 24 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2002622165 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12177059
TITLE: Regulation of kinase activity of 3-**phosphoinositide**-dependent protein kinase-1 by binding to 14-3-3.
AUTHOR: Sato Saori; Fujita Naoya; Tsuruo Takashi
CORPORATE SOURCE: Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113-0032, Japan.
SOURCE: Journal of biological chemistry, (2002 Oct 18) 277 (42) 39360-7. Electronic Publication: 2002-08-12.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20021017
Last Updated on STN: 20030105
Entered Medline: 20021219

AB 3-**Phosphoinositide**-dependent protein kinase-1 (**PDK1**) plays a central role in activating the protein kinase A, G, and C subfamily. In particular, **PDK1** plays an important role in regulating the Akt survival pathway by phosphorylating Akt on Thr-308. **PDK1** kinase activity was thought to be constitutively active; however, recent reports suggested that its activity is regulated by binding to other proteins, such as protein kinase C-related kinase-2 (**PRK2**), p90 ribosomal protein S6 kinase-2 (RSK2), and heat-shock protein 90 (Hsp90). Here we report that **PDK1** binds to 14-3-3 proteins in vivo and in vitro through the sequence surrounding Ser-241, a residue that is phosphorylated by itself and is critical for its kinase activity. Mutation of **PDK1** to increase its binding to 14-3-3 decreased its kinase activity in vivo. By contrast, mutation of **PDK1** to decrease its interaction with 14-3-3 resulted in increased **PDK1** kinase activity. Moreover, incubation of wild-type **PDK1** with recombinant 14-3-3 in vitro decreased its kinase activity. These data indicate that **PDK1** kinase activity is negatively regulated by binding to 14-3-3 through the **PDK1** autophosphorylation site Ser-241.

L6 ANSWER 11 OF 24 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002455887 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12169624
TITLE: High resolution crystal structure of the human **PDK1** catalytic domain defines the regulatory phosphopeptide docking site.
AUTHOR: Biondi Ricardo M; Komander David; Thomas Christine C; Lizcano Jose M; Deak Maria; Alessi Dario R; van Aalten Daan M F
CORPORATE SOURCE: Division of Signal Transduction Therapy, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.
SOURCE: EMBO journal, (2002 Aug 15) 21 (16) 4219-28.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1H1W
ENTRY MONTH: 200209

ENTRY DATE: Entered STN: 20020907
Last Updated on STN: 20020925
Entered Medline: 20020924

AB 3-**phosphoinositide** dependent protein kinase-1 (**PDK1**) plays a key role in regulating signalling pathways by activating AGC kinases such as PKB/Akt and S6K. Here we describe the 2.0 A crystal structure of the **PDK1** kinase domain in complex with ATP. The structure defines the hydrophobic pocket termed the "**PIF** -pocket", which plays a key role in mediating the interaction and phosphorylation of certain substrates such as S6K1. Phosphorylation of S6K1 at its C-terminal **PIF**-pocket-interacting motif promotes the binding of S6K1 with **PDK1**. In the **PDK1** structure, this pocket is occupied by a crystallographic contact with another molecule of **PDK1**. Interestingly, close to the **PIF** -pocket in **PDK1**, there is an ordered sulfate ion, interacting tightly with four surrounding side chains. The roles of these residues were investigated through a combination of site-directed mutagenesis and kinetic studies, the results of which confirm that this region of **PDK1** represents a phosphate-dependent docking site. We discuss the possibility that an analogous phosphate-binding regulatory motif may participate in the activation of other AGC kinases. Furthermore, the structure of **PDK1** provides a scaffold for the design of specific **PDK1** inhibitors.

L6 ANSWER 12 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:556441 SCISEARCH
THE GENUINE ARTICLE: 565VN
TITLE: Molecular mechanism for the regulation of protein kinase B/Akt by hydrophobic motif phosphorylation
AUTHOR: Yang J; Cron P; Thompson V; Good V M; Hess D; Hemmings B A; Barford D (Reprint)
CORPORATE SOURCE: Friedrich Miescher Inst, Maulbeerstr 66, CH-4048 Basel, Switzerland (Reprint); Friedrich Miescher Inst, CH-4048 Basel, Switzerland; Inst Canc Res, Chester Beatty Labs, Sect Struct Biol, London SW3 6JB, England
COUNTRY OF AUTHOR: Switzerland; England
SOURCE: MOLECULAR CELL, (JUN 2002) Vol. 9, No. 6, pp. 1227-1240. ISSN: 1097-2765.
PUBLISHER: CELL PRESS, 1100 MASSACHUSETTS AVE,, CAMBRIDGE, MA 02138 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 42
ENTRY DATE: Entered STN: 19 Jul 2002
Last Updated on STN: 19 Jul 2002
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Protein kinase B/Akt plays crucial roles in promoting cell survival and mediating insulin responses. The enzyme is stimulated by phosphorylation at two regulatory sites: Thr 309 of the activation segment and Ser 474 of the hydrophobic motif, a conserved feature of many AGC kinases. Analysis of the crystal structures of the unphosphorylated and Thr 309 phosphorylated states of the PKB kinase domain provides a molecular explanation for regulation by Ser 474 phosphorylation. Activation by Ser 474 phosphorylation occurs via a disorder to order transition of the alphaC helix with concomitant restructuring of the activation segment and reconfiguration of the kinase bilobal structure. These conformational changes are mediated by a phosphorylation-promoted interaction of the hydrophobic motif with a channel on the N-terminal lobe induced by the ordered alphaC helix and are mimicked by peptides corresponding to the hydrophobic motif of PKB and potently by the hydrophobic motif of **PRK2**.

L6 ANSWER 13 OF 24 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2002055627 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11781095
TITLE: Regulation of both **PDK1** and the phosphorylation of PKC-zeta and -delta by a C-terminal **PRK2** fragment.
AUTHOR: Hodgkinson Conrad P; Sale Graham J
CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Southampton, UK.
SOURCE: Biochemistry, (2002 Jan 15) 41 (2) 561-9.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020420
Entered Medline: 20020204

AB The mechanism by which **PDK1** regulates AGC kinases remains unclear. To further understand this process, we performed a yeast two-hybrid screen using **PDK1** as bait. PKC-zeta, PKC-delta, and **PRK2** were identified as interactors of **PDK1**. A combination of yeast two-hybrid binding assays and coprecipitation from mammalian cells was used to characterize the nature of the **PDK1** -PKC interaction. The presence of the PH domain of **PDK1** inhibited the interaction of **PDK1** with the PKCs. A contact region of **PDK1** was mapped between residues 314 and 408. The interaction of **PDK1** with the PKCs required the full-length PKC-zeta and -delta proteins apart from their C-terminal tails. **PDK1** was able to phosphorylate full-length PKC-zeta and -delta but not PKC-zeta and -delta constructs containing the **PDK1** phosphorylation site but lacking the C-terminal tails. A C-terminal **PRK2** fragment, normally produced by caspase-3 cleavage during apoptosis, inhibited **PDK1** autophosphorylation by >90%. The ability of **PDK1** to phosphorylate PKC-zeta and -delta in vitro was also markedly inhibited by the **PRK2** fragment. Additionally, generation of the **PRK2** fragment in vivo inhibited by >90% the phosphorylation of endogenous PKC-zeta by **PDK1**. In conclusion, these results show that the C-terminal tail of PKC is a critical determinant for PKC-zeta and -delta phosphorylation by **PDK1**. Moreover, the C-terminal **PRK2** fragment acts as a potent negative regulator of **PDK1** autophosphorylation and **PDK1** kinase activity against PKC-zeta and -delta. As the C-terminal **PRK2** fragment is naturally generated during apoptosis, this may provide a mechanism of restraining prosurvival signals during apoptosis.

L6 ANSWER 14 OF 24 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2002630189 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12387817
TITLE: The Na(+)/H(+) exchanger regulatory factor 2 mediates phosphorylation of serum- and glucocorticoid-induced protein kinase 1 by 3-**phosphoinositide**-dependent protein kinase 1.
AUTHOR: Chun Jaesun; Kwon Taegun; Lee Eunjung; Suh Pann-Ghill; Choi Eui-Ju; Sun Kang Sang
CORPORATE SOURCE: School of Science Education, Chungbuk National University, Gaeshin-dong, Heungdok-gu, Chongju 361-763, Republic of Korea.
SOURCE: Biochemical and biophysical research communications, (2002 Oct 25) 298 (2) 207-15.
Journal code: 0372516. ISSN: 0006-291X.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20021022
Last Updated on STN: 20021214
Entered Medline: 20021126

AB The Na(+)/H(+) exchanger regulatory factor 2 (NHERF2/TKA-1/E3KARP) contains two PSD-95/Dlg/ZO-1 (PDZ) domains which interact with the PDZ docking motif (X-(S/T)-X-(V/L)) of proteins to mediate the assembly of transmembrane and cytosolic proteins into functional signal transduction complexes. One of the PDZ domains of NHERF2 interacts specifically with the DSLL, DSFL, and DTRL motifs present at the carboxy-termini of the 2-adrenergic receptor, the platelet-derived growth factor receptor, and the cystic fibrosis transmembrane conductance regulator, respectively. Serum- and glucocorticoid-induced protein kinase 1 (SGK1) also carries a putative PDZ-binding motif (D-S-F-L) at its carboxy tail, implicated in the specific interaction with NHERF2. There is a 3-**phosphoinositide**-dependent protein kinase 1 (**PDK1**) interacting fragment (**PIF**) in the tail of NHERF2. Using pull-down assays and co-transfection experiments, we demonstrated that the DSFL tail of SGK1 interacts with the first PDZ domain of NHERF2 and the **PIF** of NHERF2 binds to the **PIF**-binding pocket of **PDK1** to form an SGK1-NHERF2-**PDK1** complex. Formation of the protein complex promoted the phosphorylation and activation of SGK1 by **PDK1**. Thus, it was suggested that NHERF2 mediates the activation and phosphorylation of SGK1 by **PDK1** through its first PDZ domain and **PIF** motif, as a novel SGK1 activation mechanism.

L6 ANSWER 15 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:453281 HCAPLUS
DOCUMENT NUMBER: 135:73331
TITLE: Method for identifying modulators of protein kinases **PDK1**, SGK, S6 kinase, **PRK2**, and protein kinases A, B, and C
INVENTOR(S): Alessi, Dario; Biondi, Ricardo
PATENT ASSIGNEE(S): University of Dundee, UK
SOURCE: PCT Int. Appl., 180 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001044497	A2	20010621	WO 2000-GB4598	20001204
WO 2001044497	A3	20020314		
W: AU, CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
EP 1234188	A2	20020828	EP 2000-985454	20001204
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR				
JP 2003516760	T2	20030520	JP 2001-545574	20001204
US 2003143656	A1	20030731	US 2003-148786	20030108
PRIORITY APPLN. INFO.:			US 1999-168559P P	19991202
			WO 2000-GB4598 W	20001204

AB A method of identifying a compound that modulates the protein kinase activity of a protein kinase having a hydrophobic pocket in the position equivalent to the hydrophobic pocket of Protein Kinase A (PKA) that is defined

by residues including Lys76, Leu116, Val80 and/or Lys111 of full-length mouse PKA, wherein the ability of the compound to inhibit, promote or mimic the interaction of the said hydrophobic pocket-containing protein kinase with an interacting polypeptide is measured and a compound that inhibits, promotes or mimics the said interaction is selected, wherein the interacting polypeptide interacts with the hydrophobic pocket of the protein kinase and/or comprises the amino acid sequence Phe/Tyr-Xaa-Xaa-Phe/Tyr. The protein kinase may be **PDK1**, PKB, SGK or p70 S6 kinase. A method of identifying a compound that modulates the protein kinase activity of a protein kinase having a hydrophobic pocket as defined above, for example **PDK1**, comprising the steps of (1) determining the effect of a test compound on the protein kinase activity of the said protein kinase, and/or a mutant thereof, and (2) selecting a compound capable of modulating the protein kinase activity of the said protein kinase to different extents towards (i) a substrate that binds to the said hydrophobic pocket of the said protein kinase (hydrophobic pocket-dependent substrate) and (ii) a substrate (such as PKB) that does not bind, or binds to a lesser extent than the first said substrate (hydrophobic pocket-independent substrate), to the said hydrophobic pocket of the said protein kinase. The protein kinase modulators identified may be used in treatment of cancer and diabetes.

L6 ANSWER 16 OF 24 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2001454762 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11500365
TITLE: The **PIF**-binding pocket in **PDK1** is essential for activation of S6K and SGK, but not PKB.
AUTHOR: Biondi R M; Kieloch A; Currie R A; Deak M; Alessi D R
CORPORATE SOURCE: Division of Signal Transduction Therapy, MRC Protein Phosphorylation Unit, School of Life Sciences, MSI/WTB complex, University of Dundee, Dow Street, Dundee DD1 5EH, UK.. r.m.biondi@dundee.ac.uk
SOURCE: EMBO journal, (2001 Aug 15) 20 (16) 4380-90. Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010814
Last Updated on STN: 20020420
Entered Medline: 20011025

AB PKB/Akt, S6K1 and SGK are related protein kinases activated in a PI 3-kinase-dependent manner in response to insulin/growth factors signalling. Activation entails phosphorylation of these kinases at two residues, the T-loop and the hydrophobic motif. **PDK1** activates S6K, SGK and PKB isoforms by phosphorylating these kinases at their T-loop. We demonstrate that a pocket in the kinase domain of **PDK1**, termed the '**PIF**-binding pocket', plays a key role in mediating the interaction and phosphorylation of S6K1 and SGK1 at their T-loop motif by **PDK1**. Our data indicate that prior phosphorylation of S6K1 and SGK1 at their hydrophobic motif promotes their interaction with the **PIF**-binding pocket of **PDK1** and their T-loop phosphorylation. Thus, the hydrophobic motif phosphorylation of S6K and SGK converts them into substrates that can be activated by **PDK1**. In contrast, the **PIF**-binding pocket of **PDK1** is not required for the phosphorylation of PKBalpha by **PDK1**. The **PIF**-binding pocket represents a substrate recognition site on a protein kinase that is only required for the phosphorylation of a subset of its physiological substrates.

L6 ANSWER 17 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:688348 HCAPLUS
DOCUMENT NUMBER: 133:278041
TITLE: Altered specificity of **phosphoinositide** -dependent protein kinase **PDK1** in presence of substrate consensus peptides
INVENTOR(S): Alessi, Dario; Balendran, Anudharan; Deak, Maria; Currie, Richard; Downes, Peter; Casamayor, Antonio
PATENT ASSIGNEE(S): University of Dundee, UK
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000056864	A2	20000928	WO 2000-GB1004	20000317
WO 2000056864	A3	20010118		
W: JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1165761	A2	20020102	EP 2000-911069	20000317
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002539780	T2	20021126	JP 2000-606723	20000317

PRIORITY APPLN. INFO.: GB 1999-6245 A 19990319
WO 2000-GB1004 W 20000317
OTHER SOURCE(S): MARPAT 133:278041
AB A method of altering the substrate specificity of **phosphoinositide** -dependent protein kinase 1 (**PDK1**) is provided, wherein the said **PDK1** is exposed to a polypeptide which comprises the amino acid sequence Phe/Tyr-Xaa-Xaa-Phe/Tyr-Zaa-Phe/Tyr wherein Zaa represents a neg. charged amino acid residue. The **PDK1** with altered substrate specificity is capable of phosphorylating the Ser/Thr residue in a polypeptide with an amino acid sequence corresponding to the consensus sequence Phe/Tyr-Xaa-Xaa-Phe/Tyr-Ser/Thr-Phe/Tyr. The **PDK1** with altered specificity may be useful in screening assays and for phosphorylating substrates having the above consensus sequence.

L6 ANSWER 18 OF 24 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 2001098534 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11006271
TITLE: Mechanism of phosphorylation of protein kinase B/Akt by a constitutively active 3-**phosphoinositide** -dependent protein kinase-1.
AUTHOR: Wick M J; Dong L Q; Riojas R A; Ramos F J; Liu F
CORPORATE SOURCE: Departments of Pharmacology and Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78229, USA.
CONTRACT NUMBER: DK56166 (NIDDK)
SOURCE: Journal of biological chemistry, (2000 Dec 22) 275 (51) 40400-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20020420
Entered Medline: 20010201

AB Phosphorylation of Thr(308) in the activation loop and Ser(473) at the carboxyl terminus is essential for protein kinase B (PKB/Akt) activation. However, the biochemical mechanism of the phosphorylation remains to be characterized. Here we show that expression of a constitutively active mutant of mouse 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**(A280V)) in Chinese hamster ovary cells overexpressing the insulin receptor was sufficient to induce PKB phosphorylation at Thr(308) to approximately the same extent as insulin stimulation. Phosphorylation of PKB by **PDK1**(A280V) was not affected by treatment of cells with inhibitors of phosphatidylinositol 3-kinase or by deletion of the pleckstrin homology (PH) domain of PKB. C(2)-ceramide, a cell-permeable, indirect inhibitor of PKB phosphorylation, did not inhibit **PDK1** (A280V)-catalyzed PKB phosphorylation in cells and had no effect on **PDK1** activity in vitro. On the other hand, co-expression of full-length protein kinase C-related kinase-1 (PRK1/PKN) or 2 (**PRK2**) inhibited **PDK1**(A280V)-mediated PKB phosphorylation. Replacing alanine at position 280 with valine or deletion of the PH domain enhanced **PDK1** autophosphorylation in vitro. However, deletion of the PH domain of **PDK1**(A280V) significantly reduced **PDK1**(A280V)-mediated phosphorylation of PKB in cells. In resting cells, **PDK1**(A280V) localized in the cytosol and at the plasma membrane. However, **PDK1**(A280V) lacking the PH domain localized predominantly in the cytosol. Taken together, our findings suggest that the wild-type **PDK1** may not be constitutively active in cells. In addition, activation of **PDK1** is sufficient to phosphorylate PKB at Thr(308) in the cytosol. Furthermore, the PH domain of **PDK1** may play both positive and negative roles in regulating the in vivo function of the enzyme. Finally, unlike the carboxyl-terminal fragment of **PRK2**, which has been shown to bind **PDK1** and allow the enzyme to phosphorylate PKB at both Thr(308) and Ser(473), full-length **PRK2** and its related kinase PRK1/PKN may both play negative roles in PKB-mediated downstream biological events.

L6 ANSWER 19 OF 24 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 2000396616 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10764742
TITLE: A 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) docking site is required for the phosphorylation of protein kinase Czeta (PKCzeta) and PKC-related kinase 2 by **PDK1**.
AUTHOR: Balendran A; Biondi R M; Cheung P C; Casamayor A; Deak M; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Division of Signal Transduction Therapy, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, United Kingdom.
SOURCE: Journal of biological chemistry, (2000 Jul 7) 275 (27) 20806-13.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20020420
Entered Medline: 20000816

AB Members of the AGC subfamily of protein kinases including protein kinase B, p70 S6 kinase, and protein kinase C (PKC) isoforms are activated and/or stabilized by phosphorylation of two residues, one that resides in the T-loop of the kinase domain and the other that is located C-terminal to the kinase domain in a region known as the hydrophobic motif. Atypical

PKC isoforms, such as PKCzeta, and the PKC-related kinases, like **PRK2**, are also activated by phosphorylation of their T-loop site but, instead of possessing a phosphorylatable Ser/Thr in their hydrophobic motif, contain an acidic residue. The 3-**phosphoinositide** -dependent protein kinase (**PDK1**) activates many members of the AGC subfamily of kinases in vitro, including PKCzeta and **PRK2** by phosphorylating the T-loop residue. In the present study we demonstrate that the hydrophobic motifs of PKCzeta and PKCiota, as well as PRK1 and **PRK2**, interact with the kinase domain of **PDK1**. Mutation of the conserved residues of the hydrophobic motif of full-length PKCzeta, full-length **PRK2**, or **PRK2** lacking its N-terminal regulatory domain abolishes or significantly reduces the ability of these kinases to interact with **PDK1** and to become phosphorylated at their T-loop sites in vivo. Furthermore, overexpression of the hydrophobic motif of **PRK2** in cells prevents the T-loop phosphorylation and thus inhibits the activation of **PRK2** and PKCzeta. These findings indicate that the hydrophobic motif of **PRK2** and PKCzeta acts as a "docking site" enabling the recruitment of **PDK1** to these substrates. This is essential for their phosphorylation by **PDK1** in cells.

L6 ANSWER 20 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:270248 HCAPLUS
DOCUMENT NUMBER: 133:70575
TITLE: Rho GTPase control of protein kinase C-related protein kinase activation by 3-**phosphoinositide** -dependent protein kinase
AUTHOR(S): Flynn, Peter; Mellor, Harry; Casamassima, Adele; Parker, Peter J.
CORPORATE SOURCE: Imperial Cancer Research Fund, Protein Phosphorylation Laboratory, London, WC2A 3PX, UK
SOURCE: Journal of Biological Chemistry (2000), 275(15), 11064-11070
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The protein kinase C-related protein kinases (PRKs) have been shown to be under the control of the Rho GTPases and influenced by autophosphorylation. In analyzing the relationship between these inputs, it is shown that activation in vitro and in vivo involves the activation loop phosphorylation of PRK1/2 by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**). Rho overexpression in cultured cells is shown to increase the activation loop phosphorylation of endogenous PRKs and is demonstrated to influence this process by controlling the ability of PRKs to bind to **PDK1**. The interaction of PRK1/2 with **PDK1** is shown to be dependent upon Rho. Direct demonstration of ternary (Rho·PRK·**PDK1**) complex formation in situ is provided by the observation that **PDK1** is recruited to RhoB-containing endosomes only if PRK is coexpressed. Furthermore, this in vivo complex is maintained after **phosphoinositide** 3-kinase inhibition. The control of PRKs by **PDK1** thus evidences a novel strategy of substrate-directed control involving GTPases.
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 21 OF 24 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 2000164465 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10698939
TITLE: Identification of a pocket in the **PDK1** kinase domain that interacts with **PIF** and the C-terminal residues of PKA.

AUTHOR: Biondi R M; Cheung P C; Casamayor A; Deak M; Currie R A; Alessi D R
CORPORATE SOURCE: Divison of Signal Transduction Therapy, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, UK.. rbiondi@bad.dundee.ac.uk
SOURCE: EMBO journal, (2000 Mar 1) 19 (5) 979-88.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20020420
Entered Medline: 20000426

AB The 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) phosphorylates and activates a number of protein kinases of the AGC subfamily. The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (**PRK2**), termed the **PDK1**-interacting fragment (**PIF**), through a hydrophobic motif. Here we identify a hydrophobic pocket in the small lobe of the **PDK1** kinase domain, separate from the ATP- and substrate-binding sites, that interacts with **PIF**. Mutation of residues predicted to form part of this hydrophobic pocket either abolished or significantly diminished the affinity of **PDK1** for **PIF**. **PIF** increased the rate at which **PDK1** phosphorylated a synthetic dodecapeptide (T308tide), corresponding to the sequences surrounding the **PDK1** phosphorylation site of PKB. This peptide is a poor substrate for **PDK1**, but a peptide comprising T308tide fused to the **PDK1**-binding motif of **PIF** was a vastly superior substrate for **PDK1**. Our results suggest that the **PIF**-binding pocket on the kinase domain of **PDK1** acts as a 'docking site', enabling it to interact with and enhance the phosphorylation of its substrates.

L6 ANSWER 22 OF 24 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 2001061082 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11078882
TITLE: Further evidence that 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) is required for the stability and phosphorylation of protein kinase C (PKC) isoforms.
AUTHOR: Balendran A; Hare G R; Kieloch A; Williams M R; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation, MSI/WTB complex, University of Dundee, Dow Street, DD1 5EH, Dundee, UK.
SOURCE: FEBS letters, (2000 Nov 10) 484 (3) 217-23.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20020420
Entered Medline: 20001222

AB The multi-site phosphorylation of the protein kinase C (PKC) superfamily plays an important role in the regulation of these enzymes. One of the key phosphorylation sites required for the activation of all PKC isoforms lies in the T-loop of the kinase domain. Recent in vitro and transfection experiments indicate that phosphorylation of this residue can be mediated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**). In this study, we demonstrate that in embryonic stem (ES) cells lacking **PDK1** (**PDK1**-/- cells), the intracellular

levels of endogenously expressed PKCalpha, PKCbetaI, PKCgamma, PKCdelta, PKCepsilon, and PKC-related kinase-1 (PRK1) are vastly reduced compared to control ES cells (**PDK1**+/+ cells). The levels of PKCzeta and **PRK2** protein are only moderately reduced in the **PDK1**-/- ES cells. We demonstrate that in contrast to PKCzeta expressed **PDK1**+/+ ES cells, PKCzeta in ES cells lacking **PDK1** is not phosphorylated at its T-loop residue. This provides the first genetic evidence that PKCzeta is a physiological substrate for **PDK1**. In contrast, **PRK2** is still partially phosphorylated at its T-loop in **PDK1**-/- cells, indicating the existence of a **PDK1** -independent mechanism for the phosphorylation of **PRK2** at this residue.

L6 ANSWER 23 OF 24 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 2000069735 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10601311
TITLE: Evidence that 3-**phosphoinositide**-dependent protein kinase-1 mediates phosphorylation of p70 S6 kinase in vivo at Thr-412 as well as Thr-252.
AUTHOR: Balendran A; Currie R; Armstrong C G; Avruch J; Alessi D R
CORPORATE SOURCE: Medical Research Council Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, Scotland.
SOURCE: Journal of biological chemistry, (1999 Dec 24) 274 (52) 37400-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20020420
Entered Medline: 20000113

AB Protein kinase B and p70 S6 kinase are members of the cyclic AMP-dependent/cyclic GMP-dependent/protein kinase C subfamily of protein kinases and are activated by a phosphatidylinositol 3-kinase-dependent pathway when cells are stimulated with insulin or growth factors. Both of these kinases are activated in cells by phosphorylation of a conserved residue in the kinase domain (Thr-308 of protein kinase B (PKB) and Thr-252 of p70 S6 kinase) and another conserved residue located C-terminal to the kinase domain (Ser-473 of PKB and Thr-412 of p70 S6 kinase). Thr-308 of PKBalpha and Thr-252 of p70 S6 kinase are phosphorylated by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) in vitro. Recent work has shown that **PDK1** interacts with a region of protein kinase C-related kinase-2, termed the **PDK1** interacting fragment (**PIF**). Interaction with **PIF** converts **PDK1** from a form that phosphorylates PKB at Thr-308 alone to a species capable of phosphorylating Ser-473 as well as Thr-308. This suggests that **PDK1** may be the enzyme that phosphorylates both residues in vivo. Here we demonstrate that **PDK1** is capable of phosphorylating p70 S6 kinase at Thr-412 in vitro. We study the effect of **PIF** on the ability of **PDK1** to phosphorylate p70 S6 kinase. Surprisingly, we find that **PDK1** bound to **PIF** is no longer able to interact with or phosphorylate p70 S6 kinase in vitro at either Thr-252 or Thr-412. The expression of **PIF** in cells prevents insulin-like growth factor 1 from inducing the activation of the p70 S6 kinase and its phosphorylation at Thr-412. Overexpression of **PDK1** in cells induces the phosphorylation of p70 S6 kinase at Thr-412 in unstimulated cells, and a catalytically inactive mutant of **PDK1** prevents the phosphorylation of p70 S6K at Thr-412 in insulin-like growth factor 1-stimulated cells. These observations indicate that **PDK1** regulates the activation of p70 S6 kinase and

provides evidence that **PDK1** mediates the phosphorylation of p70 S6 kinase at Thr-412.

L6 ANSWER 24 OF 24 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 1999244939 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10226025
TITLE: **PDK1** acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of **PRK2**.
AUTHOR: Balendran A; Casamayor A; Deak M; Paterson A; Gaffney P; Currie R; Downes C P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK.
SOURCE: Current biology : CB, (1999 Apr 22) 9 (8) 393-404. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20020420
Entered Medline: 19990601

AB BACKGROUND: Protein kinase B (PKB) is activated by phosphorylation of Thr308 and of Ser473. Thr308 is phosphorylated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) but the identity of the kinase that phosphorylates Ser473 (provisionally termed PDK2) is unknown. RESULTS: The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (**PRK2**), termed the **PDK1**-interacting fragment (**PIF**). **PIF** is situated carboxy-terminal to the kinase domain of **PRK2**, and contains a consensus motif for phosphorylation by PDK2 similar to that found in PKBalpha, except that the residue equivalent to Ser473 is aspartic acid. Mutation of any of the conserved residues in the PDK2 motif of **PIF** prevented interaction of **PIF** with **PDK1**. Remarkably, interaction of **PDK1** with **PIF**, or with a synthetic peptide encompassing the PDK2 consensus sequence of **PIF**, converted **PDK1** from an enzyme that could phosphorylate only Thr308 of PKBalpha to one that phosphorylates both Thr308 and Ser473 of PKBalpha in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3). Furthermore, the interaction of **PIF** with **PDK1** converted the **PDK1** from a form that is not directly activated by PtdIns(3,4,5)P3 to a form that is activated threefold by PtdIns(3,4,5)P3. We have partially purified a kinase from brain extract that phosphorylates Ser473 of PKBalpha in a PtdIns(3,4,5)P3-dependent manner and that is immunoprecipitated with **PDK1** antibodies. CONCLUSIONS: **PDK1** and PDK2 might be the same enzyme, the substrate specificity and activity of **PDK1** being regulated through its interaction with another protein(s). **PRK2** is a probable substrate for **PDK1**.

=> d his

(FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005
L1 1799 S "PDK1"
L2 62622 S PHOSPHOINOSITIDE##
L3 1051 S L1 AND L2
L4 2934 S "PIF" OR "PRK2"

```
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7

=> s l3 and PKBalpha
L9            35 L3 AND PKBALPHA

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)

=> d 1-19 ibib ab

L10  ANSWER 1 OF 19  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation  on
     STN
ACCESSION NUMBER:     2004:296280  SCISEARCH
THE GENUINE ARTICLE:  804YV
TITLE:                Neuregulin signaling on glucose transport in muscle cells
AUTHOR:               Canto C; Suarez E; Lizcano J M; Grino E; Shepherd P R;
                      Fryer L G D; Carling D; Bertran J; Palacin M; Zorzano A
                      (Reprint); Guma A
CORPORATE SOURCE:     Univ Barcelona, Dept Bioquim & Biol Mol, Avda Diagonal
                      645, E-08028 Barcelona, Spain (Reprint); Univ Barcelona,
                      Dept Bioquim & Biol Mol, E-08028 Barcelona, Spain; Univ
                      Barcelona, Parc Cient Barcelona, E-08028 Barcelona, Spain;
                      Univ Dundee, Sch Life Sci, MRC, Prot Phosphorylat Unit,
                      Dundee DD1 4HN, Scotland; Univ London Univ Coll, Dept
                      Biochem, London WC1E 6BT, England; Hammersmith Hosp,
                      Imperial Coll Med, Sch Med, MRC, Clin Sci Ctr, Cellular
                      Stress Grp, London W12 0NN, England
COUNTRY OF AUTHOR:    Spain; Scotland; England
SOURCE:               JOURNAL OF BIOLOGICAL CHEMISTRY, (26 MAR 2004) Vol. 279,
                      No. 13, pp. 12260-12268.
                      ISSN: 0021-9258.
PUBLISHER:            AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650
                      ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
DOCUMENT TYPE:        Article; Journal
LANGUAGE:             English
REFERENCE COUNT:      62
ENTRY DATE:           Entered STN: 9 Apr 2004
                      Last Updated on STN: 9 Apr 2004
                     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
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AB Neuregulin-1, a growth factor that potentiates myogenesis induces glucose transport through translocation of glucose transporters, in an additive manner to insulin, in muscle cells. In this study, we examined the signaling pathway required for a recombinant active neuregulin-1 isoform (rhHeregulin-beta(1), 177-244, HRG) to stimulate glucose uptake in L6E9 myotubes. The stimulatory effect of HRG required binding to ErbB3 in L6E9 myotubes. PI3K activity is required for HRG action in both muscle cells and tissue. In L6E9 myotubes, HRG stimulated **PKBalpha**, PKBgamma, and PKCzeta activities. TPCK, an inhibitor of **PDK1**, abolished both HRG- and insulin-induced glucose transport. To assess whether PKB was necessary for the effects of HRG on glucose uptake, cells were infected with adenoviruses encoding dominant negative mutants of **PKBalpha**. Dominant negative PKB reduced PKB activity and insulin-stimulated glucose transport but not HRG- induced glucose transport. In contrast, transduction of L6E9 myotubes with adenoviruses encoding a dominant negative kinase-inactive PKCzeta abolished both HRG- and insulin-stimulated glucose uptake. In soleus muscle, HRG induced PKCzeta, but not PKB phosphorylation. HRG also stimulated the activity of p70S6K, p38MAPK, and p42/p44MAPK and inhibition of p42/p44MAPK partially repressed HRG action on glucose uptake. HRG did not affect AMPKalpha(1)

or AMPKalpha(2) activities. In all, HRG stimulated glucose transport in muscle cells by activation of a pathway that requires PI3K, **PDK1**, and PKCzeta, but not PKB, and that shows cross-talk with the MAPK pathway. The PI3K, **PDK1**, and PKCzeta pathway can be considered as an alternative mechanism, independent of insulin, to induce glucose uptake.

L10 ANSWER 2 OF 19 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2004259252 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15157674
TITLE: Regulation of protein kinase B/Akt activity and Ser473 phosphorylation by protein kinase Calpha in endothelial cells.
AUTHOR: Partovian Chohreh; Simons Michael
CORPORATE SOURCE: Department of Medicine, Angiogenesis Research Center and Section of Cardiology, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756, USA.
CONTRACT NUMBER: HL62289 (NHLBI)
HL63609 (NHLBI)
SOURCE: Cellular signalling, (2004 Aug) 16 (8) 951-7.
Journal code: 8904683. ISSN: 0898-6568.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200505
ENTRY DATE: Entered STN: 20040526
Last Updated on STN: 20050520
Entered Medline: 20050519
AB Protein kinase Balpha (**PKBalpha**/Akt-1) is a key mediator of multiple signaling pathways involved in angiogenesis, cell proliferation and apoptosis among others. The unphosphorylated form of Akt-1 is virtually inactive and its full activation requires two phosphatidylinositol-3,4,5-triphosphate-dependent phosphorylation events, Thr308 by 3-**phosphoinositide**-dependent kinase-1 (**PDK1**) and Ser473 by an undefined kinase that has been termed PDK2. Recent studies have suggested that the Ser473 kinase is a plasma membrane raft-associated kinase. In this study we show that protein kinase Calpha (PKCalpha) translocates to the membrane rafts in response to insulin growth factor-1 (IGF-1) stimulation. Overexpression of PKCalpha increases Ser473 phosphorylation and Akt-1 activity, while inhibition of its activity or expression decreases IGF-1-dependent activation of Akt-1. Furthermore, in vitro, in the presence of phospholipids and calcium, PKCalpha directly phosphorylates Akt-1 at the Ser473 site. We conclude, therefore, that PKCalpha regulates Akt-1 activity via Ser473 phosphorylation and may function as PDK2 in endothelial cells.

L10 ANSWER 3 OF 19 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2004523603 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15494023
TITLE: Analysis of insulin signalling by RNAi-based gene silencing.
AUTHOR: Zhou Q L; Park J G; Jiang Z Y; Holik J J; Mitra P; Semiz S; Guilherme A; Powelka A M; Tang X; Virbasius J; Czech M P
CORPORATE SOURCE: Program in Molecular Medicine, University of Massachusetts Medical School, 373 Plantation Street, Worcester, MA 01605, USA.
CONTRACT NUMBER: 5 P30 DK32520 (NIDDK)
DK30648 (NIDDK)
DK30898 (NIDDK)
DK60837 (NIDDK)
SOURCE: Biochemical Society transactions, (2004 Nov) 32 (Pt 5)

817-21. Ref: 35
Journal code: 7506897. ISSN: 0300-5127.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200504
ENTRY DATE: Entered STN: 20041022
Last Updated on STN: 20050409
Entered Medline: 20050408

AB Using siRNA-mediated gene silencing in cultured adipocytes, we have dissected the insulin-signalling pathway leading to translocation of GLUT4 glucose transporters to the plasma membrane. RNAi (RNA interference)-based depletion of components in the putative TC10 pathway (CAP, CrkII and c-Cbl plus Cbl-b) or the phospholipase Cgamma pathway failed to diminish insulin signalling to GLUT4. Within the **phosphoinositide** 3-kinase pathway, loss of the 5'-phosphatidylinositol 3,4,5-trisphosphate phosphatase SHIP2 was also without effect, whereas depletion of the 3'-phosphatase PTEN significantly enhanced insulin action. Downstream of phosphatidylinositol 3,4,5-trisphosphate and **PDK1**, silencing the genes encoding the protein kinases Akt1/**PKBalpha**, or CISK(SGK3) or protein kinases Clambda/zeta had little or no effect, but loss of Akt2/PKBbeta significantly attenuated GLUT4 regulation by insulin. These results show that Akt2/PKBbeta is the key downstream intermediate within the **phosphoinositide** 3-kinase pathway linked to insulin action on GLUT4 in cultured adipocytes, whereas PTEN is a potent negative regulator of this pathway.

L10 ANSWER 4 OF 19 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2004600866 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15461588
TITLE: Identification of filamin C as a new physiological substrate of **PKBalpha** using KESTREL.
AUTHOR: Murray James T; Campbell David G; Peggie Mark; Alfonso Mora; Cohen Philip
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.. j.t.c.murray@dundee.ac.uk
SOURCE: Biochemical journal, (2004 Dec 15) 384 (Pt 3) 489-94.
Journal code: 2984726R. ISSN: 1470-8728.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200505
ENTRY DATE: Entered STN: 20041203
Last Updated on STN: 20050512
Entered Medline: 20050511

AB We detected a protein in rabbit skeletal muscle extracts that was phosphorylated rapidly by PKBa (protein kinase Ba), but not by SGK1 (serum- and glucocorticoid-induced kinase 1), and identified it as the cytoskeletal protein FLNc (filamin C). PKBa phosphorylated FLNc at Ser2213 in vitro, which lies in an insert not present in the FLNa and FLNb isoforms. Ser2213 became phosphorylated when C2C12 myoblasts were stimulated with insulin or epidermal growth factor, and phosphorylation was prevented by low concentrations of wortmannin, at which it is a relatively specific inhibitor of **phosphoinositide** 3-kinase. PD 184352 [an inhibitor of the classical MAPK (mitogen-activated protein kinase) cascade] and/or rapamycin [an inhibitor of mTOR (mammalian target of rapamycin)] had no effect. Insulin also induced the phosphorylation of

FLNc at Ser2213 in cardiac muscle in vivo, but not in cardiac muscle that does not express **PDK1** (3-**phosphoinositide**-dependent kinase 1), the upstream activator of PKB. These results identify the muscle-specific isoform FLNc as a new physiological substrate for PKB.

L10 ANSWER 5 OF 19 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2003493613 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12964941
TITLE: Binding of phosphatidylinositol 3,4,5-trisphosphate to the pleckstrin homology domain of protein kinase B induces a conformational change.
AUTHOR: Milburn Christine C; Deak Maria; Kelly Sharon M; Price Nick C; Alessi Dario R; Van Aalten Daan M F
CORPORATE SOURCE: Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.
SOURCE: Biochemical journal, (2003 Nov 1) 375 (Pt 3) 531-8. Journal code: 2984726R. ISSN: 1470-8728.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1UNP; PDB-1UNQ; PDB-1UNR
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 20031023
Last Updated on STN: 20040427
Entered Medline: 20040426

AB Protein kinase B (PKB/Akt) is a key regulator of cell growth, proliferation and metabolism. It possesses an N-terminal pleckstrin homology (PH) domain that interacts with equal affinity with the second messengers PtdIns(3,4,5)P3 and PtdIns(3,4)P2, generated through insulin and growth factor-mediated activation of **phosphoinositide** 3-kinase (PI3K). The binding of PKB to PtdIns(3,4,5)P3/PtdIns(3,4)P2 recruits PKB from the cytosol to the plasma membrane and is also thought to induce a conformational change that converts PKB into a substrate that can be activated by the **phosphoinositide**-dependent kinase 1 (**PDK1**). In this study we describe two high-resolution crystal structures of the PH domain of **PKBalpha** in a noncomplexed form and compare this to a new atomic resolution (0.98 A, where 1 A=0.1 nm) structure of the PH domain of **PKBalpha** complexed to Ins(1,3,4,5)P4, the head group of PtdIns(3,4,5)P3. Remarkably, in contrast to all other PH domains crystallized so far, our data suggest that binding of Ins(1,3,4,5)P4 to the PH domain of PKB, induces a large conformational change. This is characterized by marked changes in certain residues making up the **phosphoinositide**-binding site, formation of a short a-helix in variable loop 2, and a movement of variable loop 3 away from the lipid-binding site. Solution studies with CD also provided evidence of conformational changes taking place upon binding of Ins(1,3,4,5)P4 to the PH domain of PKB. Our data provides the first structural insight into the mechanism by which the interaction of PKB with PtdIns(3,4,5)P3/PtdIns(3,4)P2 induces conformational changes that could enable PKB to be activated by **PDK1**.

L10 ANSWER 6 OF 19 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002204816 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11825911
TITLE: Protein kinase B is regulated in platelets by the collagen receptor glycoprotein VI.
AUTHOR: Barry Fiona A; Gibbins Jonathan M
CORPORATE SOURCE: School of Animal & Microbial Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, United Kingdom.
SOURCE: Journal of biological chemistry, (2002 Apr 12) 277 (15) 12874-8. Electronic Publication: 2002-02-01.

Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020409
Last Updated on STN: 20030105
Entered Medline: 20020516

AB **Phosphoinositide** 3-kinase (PI3K) is a critical component of the signaling pathways that control the activation of platelets. Here we have examined the regulation of protein kinase B (PKB), a downstream effector of PI3K, by the platelet collagen receptor glycoprotein (GP) VI and thrombin receptors. Stimulation of platelets with collagen or convulxin (a selective GPVI agonist) resulted in PI3K-dependent, and aggregation independent, Ser(473) and Thr(308) phosphorylation of **PKBalpha**, which results in PKB activation. This was accompanied by translocation of PKB to cell membranes. The **phosphoinositide**-dependent kinase **PDK1** is known to phosphorylate **PKBalpha** on Thr(308), although the identity of the kinase responsible for Ser(473) phosphorylation is less clear. One candidate that has been implicated as being responsible for Ser(473) phosphorylation, either directly or indirectly, is the integrin-linked kinase (ILK). In this study we have examined the interactions of PKB, **PDK1**, and ILK in resting and stimulated platelets. We demonstrate that in platelets PKB is physically associated with **PDK1** and ILK. Furthermore, the association of **PDK1** and ILK increases upon platelet stimulation. It would therefore appear that formation of a tertiary complex between **PDK1** , ILK, and PKB may be necessary for phosphorylation of PKB. These observations indicate that PKB participates in cell signaling downstream of the platelet collagen receptor GPVI. The role of PKB in collagen- and thrombin-stimulated platelets remains to be determined.

L10 ANSWER 7 OF 19 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2002658121 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12374740
TITLE: A phosphoserine/threonine-binding pocket in AGC kinases and **PDK1** mediates activation by hydrophobic motif phosphorylation.
AUTHOR: Frodin Morten; Antal Torben L; Dummler Bettina A; Jensen Claus J; Deak Maria; Gammeltoft Steen; Biondi Ricardo M
CORPORATE SOURCE: Department of Clinical Biochemistry, Glostrup Hospital, DK-2600 Glostrup, Denmark.. mf@dcb-glostrup.dk
SOURCE: EMBO journal, (2002 Oct 15) 21 (20) 5396-407.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20021107
Last Updated on STN: 20021214
Entered Medline: 20021126

AB The growth factor-activated AGC protein kinases RSK, S6K, PKB, MSK and SGK are activated by serine/threonine phosphorylation in the activation loop and in the hydrophobic motif, C-terminal to the kinase domain. In some of these kinases, phosphorylation of the hydrophobic motif creates a specific docking site that recruits and activates **PDK1**, which then phosphorylates the activation loop. Here, we discover a pocket in the kinase domain of **PDK1** that recognizes the phosphoserine/phosphothreonine in the hydrophobic motif by identifying two oppositely positioned arginine and lysine residues that bind the phosphate. Moreover, we demonstrate that RSK2, S6K1, **PKBalpha**,

MSK1 and SGK1 contain a similar phosphate-binding pocket, which they use for intramolecular interaction with their own phosphorylated hydrophobic motif. Molecular modelling and experimental data provide evidence for a common activation mechanism in which the phosphorylated hydrophobic motif and activation loop act on the alphaC-helix of the kinase structure to induce synergistic stimulation of catalytic activity. Sequence conservation suggests that this mechanism is a key feature in activation of >40 human AGC kinases.

L10 ANSWER 8 OF 19 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:468641 BIOSIS
DOCUMENT NUMBER: PREV200200468641
TITLE: High-resolution structure of the pleckstrin homology domain of protein kinase B/Akt bound to phosphatidylinositol (3,4,5)-trisphosphate.
AUTHOR(S): Thomas, Christine C.; Deak, Maria; Alessi, Dario R.; van Aalten, Daan M. F. [Reprint author]
CORPORATE SOURCE: Division of Biological Chemistry and Molecular Microbiology, University of Dundee, Dundee, DD1 5EH, UK dava@davapc1.bioch.dundee.ac.uk
SOURCE: Current Biology, (July 23, 2002) Vol. 12, No. 14, pp. 1256-1262. print.
CODEN: CUBLE2. ISSN: 0960-9822.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Sep 2002
Last Updated on STN: 4 Sep 2002

AB The products of PI 3-kinase activation, PtdIns(3,4,5)P3 and its immediate breakdown product PtdIns(3,4)P2, trigger physiological processes, by interacting with proteins possessing pleckstrin homology (PH) domains. One of the best characterized PtdIns(3,4,5)P3/PtdIns(3,4)P2 effector proteins is protein kinase B (PKB), also known as Akt. PKB possesses a PH domain located at its N terminus, and this domain binds specifically to PtdIns(3,4,5)P3 and PtdIns(3,4)P2 with similar affinity. Following activation of PI 3-kinase, PKB is recruited to the plasma membrane by virtue of its interaction with PtdIns(3,4,5)P3/PtdIns(3,4)P2. PKB is then activated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**), which like PKB, possesses a PtdIns(3,4,5)P3/PtdIns(3,4)P2 binding PH domain. Here, we describe the high-resolution crystal structure of the isolated PH domain of **PKBalpha** in complex with the head group of PtdIns(3,4,5)P3. The head group has a significantly different orientation and location compared to other Ins(1,3,4,5)P4 binding PH domains. Mutagenesis of the basic residues that form ionic interactions with the D3 and D4 phosphate groups reduces or abolishes the ability of PKB to interact with PtdIns(3,4,5)P3 and PtdIns(3,4)P2. The D5 phosphate faces the solvent and forms no significant interactions with any residue on the PH domain, and this explains why PKB interacts with similar affinity with both PtdIns(3,4,5)P3 and PtdIns(3,4)P2.

L10 ANSWER 9 OF 19 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:409002 BIOSIS
DOCUMENT NUMBER: PREV200100409002
TITLE: Insulin-stimulated protein kinase B phosphorylation on Ser-473 is independent of its activity and occurs through a staurosporine-insensitive kinase.
AUTHOR(S): Hill, Michelle M.; Andjelkovic, Mirjana; Brazil, Derek P.; Ferrari, Stefano; Fabbro, Doriano; Hemmings, Brian A. [Reprint author]
CORPORATE SOURCE: Friedrich Miescher Institute, Maulbeerstrasse 66, CH-4058, Basel, Switzerland
SOURCE: Journal of Biological Chemistry, (July 13, 2001) Vol. 276, No. 28, pp. 25643-25646. print.
CODEN: JBCHA3. ISSN: 0021-9258.

DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 29 Aug 2001
Last Updated on STN: 22 Feb 2002

AB Full activation of protein kinase B (PKB, also called Akt) requires phosphorylation on two regulatory sites, Thr-308 in the activation loop and Ser-473 in the hydrophobic C-terminal regulatory domain (numbering for **PKBalpha**/Akt-1). Although 3'-**phosphoinositide**-dependent protein kinase 1 (**PDK1**) has now been identified as the Thr-308 kinase, the mechanism of the Ser-473 phosphorylation remains controversial. As a step to further characterize the Ser-473 kinase, we examined the effects of a range of protein kinase inhibitors on the activation and phosphorylation of PKB. We found that staurosporine, a broad-specificity kinase inhibitor and inducer of cell apoptosis, attenuated PKB activation exclusively through the inhibition of Thr-308 phosphorylation, with Ser-473 phosphorylation unaffected. The increase in Thr-308 phosphorylation because of overexpression of **PDK1** was also inhibited by staurosporine. We further show that staurosporine (CGP 39360) potently inhibited **PDK1** activity in vitro with an IC50 of apprx0.22 muM. These data indicate that agonist-induced phosphorylation of Ser-473 of PKB is independent of **PDK1** or PKB activity and occurs through a distinct Ser-473 kinase that is not inhibited by staurosporine. Moreover, our results suggest that inhibition of PKB signaling is involved in the proapoptotic action of staurosporine.

L10 ANSWER 10 OF 19 MEDLINE on STN
ACCESSION NUMBER: 2001409989 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11313398
TITLE: Fc alpha receptor cross-linking causes translocation of phosphatidylinositol-dependent protein kinase 1 and protein kinase B alpha to MHC class II peptide-loading-like compartments.
AUTHOR: Lang M L; Shen L; Gao H; Cusack W F; Lang G A; Wade W F
CORPORATE SOURCE: Department of Microbiology, Dartmouth Medical School, Lebanon, NH 03756, USA.
CONTRACT NUMBER: RO1AI22816 (NIAID)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2001 May 1) 166 (9) 5585-93.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20021219
Entered Medline: 20010719

AB A20 IIA1.6 B cells cotransfected with FcalphaR and wild-type gamma-chain (wt-ITAM (immunoreceptor tyrosine-based activation motif)) or FcalphaR and gamma-chain, in which the wt-ITAM was substituted with the FcgammaRIIA ITAM (IIA-ITAM), were used to investigate cell signaling events influencing presentation of FcalphaR-targeted exogenous Ag in the context of MHC class II. wt-ITAM cells presented FcalphaR-targeted OVA more efficiently than IIA-ITAM transfectants to OVA-specific T cell hybridomas. Phosphatidylinositol 3-kinase (PI 3-kinase) inhibition abrogated Ag presentation, suggesting that FcalphaR may trigger a PI 3-kinase-dependent signal transduction pathway, and thus phosphatidylinositol-dependent protein kinase (**PDK1**) and protein kinase B alpha (**PKBalpha**) activation. Cross-linking FcalphaR on wt-ITAM or IIA-ITAM cells triggered equivalent PI 3-kinase-dependent activation of **PKBalpha**. Furthermore, FcalphaR cross-linking triggered recruitment of **PDK1** and serine-phosphorylated **PKBalpha** to capped cell surface FcalphaR irrespective of the gamma-chain ITAM.

Although FcalphaR endocytosis was accompanied by translocation of **PDK1** and phospho-**PKBalpha** to FcalphaR-containing vesicles in both transfectants, this was decreased in IIA-ITAM cells, and a significant proportion of **PDK1** and **PKBalpha** remained at the plasma membrane. In wt-ITAM cells, **PDK1** and serine-phosphorylated **PKBalpha** translocated to lysosomal-associated membrane glycoprotein 1- and cathepsin B-containing vesicles, consistent with MHC class II peptide-loading compartments (MIIC) described by other groups. Our data indicate that translocation of signal transduction mediators to MIIC-like compartments accompanies efficient presentation of receptor-targeted Ag, and suggest a mechanism connecting signaling to the Ag-processing pathway.

L10 ANSWER 11 OF 19 MEDLINE on STN
ACCESSION NUMBER: 2001454762 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11500365
TITLE: The PIF-binding pocket in **PDK1** is essential for activation of S6K and SGK, but not PKB.
AUTHOR: Biondi R M; Kieloch A; Currie R A; Deak M; Alessi D R
CORPORATE SOURCE: Division of Signal Transduction Therapy, MRC Protein Phosphorylation Unit, School of Life Sciences, MSI/WTB complex, University of Dundee, Dow Street, Dundee DD1 5EH, UK.. r.m.biondi@dundee.ac.uk
SOURCE: EMBO journal, (2001 Aug 15) 20 (16) 4380-90. Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010814
Last Updated on STN: 20020420
Entered Medline: 20011025

AB PKB/Akt, S6K1 and SGK are related protein kinases activated in a PI 3-kinase-dependent manner in response to insulin/growth factors signalling. Activation entails phosphorylation of these kinases at two residues, the T-loop and the hydrophobic motif. **PDK1** activates S6K, SGK and PKB isoforms by phosphorylating these kinases at their T-loop. We demonstrate that a pocket in the kinase domain of **PDK1** , termed the 'PIF-binding pocket', plays a key role in mediating the interaction and phosphorylation of S6K1 and SGK1 at their T-loop motif by **PDK1**. Our data indicate that prior phosphorylation of S6K1 and SGK1 at their hydrophobic motif promotes their interaction with the PIF-binding pocket of **PDK1** and their T-loop phosphorylation. Thus, the hydrophobic motif phosphorylation of S6K and SGK converts them into substrates that can be activated by **PDK1**. In contrast, the PIF-binding pocket of **PDK1** is not required for the phosphorylation of **PKBalpha** by **PDK1**. The PIF-binding pocket represents a substrate recognition site on a protein kinase that is only required for the phosphorylation of a subset of its physiological substrates.

L10 ANSWER 12 OF 19 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2000069735 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10601311
TITLE: Evidence that 3-**phosphoinositide**-dependent protein kinase-1 mediates phosphorylation of p70 S6 kinase in vivo at Thr-412 as well as Thr-252.
AUTHOR: Balendran A; Currie R; Armstrong C G; Avruch J; Alessi D R
CORPORATE SOURCE: Medical Research Council Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, Scotland.
SOURCE: Journal of biological chemistry, (1999 Dec 24) 274 (52)

37400-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20020420
Entered Medline: 20000113

AB Protein kinase B and p70 S6 kinase are members of the cyclic AMP-dependent/cyclic GMP-dependent/protein kinase C subfamily of protein kinases and are activated by a phosphatidylinositol 3-kinase-dependent pathway when cells are stimulated with insulin or growth factors. Both of these kinases are activated in cells by phosphorylation of a conserved residue in the kinase domain (Thr-308 of protein kinase B (PKB) and Thr-252 of p70 S6 kinase) and another conserved residue located C-terminal to the kinase domain (Ser-473 of PKB and Thr-412 of p70 S6 kinase). Thr-308 of **PKBalpha** and Thr-252 of p70 S6 kinase are phosphorylated by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) in vitro. Recent work has shown that **PDK1** interacts with a region of protein kinase C-related kinase-2, termed the **PDK1** interacting fragment (PIF). Interaction with PIF converts **PDK1** from a form that phosphorylates PKB at Thr-308 alone to a species capable of phosphorylating Ser-473 as well as Thr-308. This suggests that **PDK1** may be the enzyme that phosphorylates both residues in vivo. Here we demonstrate that **PDK1** is capable of phosphorylating p70 S6 kinase at Thr-412 in vitro. We study the effect of PIF on the ability of **PDK1** to phosphorylate p70 S6 kinase. Surprisingly, we find that **PDK1** bound to PIF is no longer able to interact with or phosphorylate p70 S6 kinase in vitro at either Thr-252 or Thr-412. The expression of PIF in cells prevents insulin-like growth factor 1 from inducing the activation of the p70 S6 kinase and its phosphorylation at Thr-412. Overexpression of **PDK1** in cells induces the phosphorylation of p70 S6 kinase at Thr-412 in unstimulated cells, and a catalytically inactive mutant of **PDK1** prevents the phosphorylation of p70 S6K at Thr-412 in insulin-like growth factor 1-stimulated cells. These observations indicate that **PDK1** regulates the activation of p70 S6 kinase and provides evidence that **PDK1** mediates the phosphorylation of p70 S6 kinase at Thr-412.

L10 ANSWER 13 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1999287923 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10358075
TITLE: Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B.
AUTHOR: Rena G; Guo S; Cichy S C; Unterman T G; Cohen P
CORPORATE SOURCE: Department of Biochemistry, Medical Research Council Protein Phosphorylation Unit, University of Dundee, Dundee DD1 5EH, Scotland, United Kingdom.
SOURCE: Journal of biological chemistry, (1999 Jun 11) 274 (24) 17179-83.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990715
Last Updated on STN: 20020420
Entered Medline: 19990706

AB Protein kinase B lies "downstream" of phosphatidylinositide (PtdIns) 3-kinase and is thought to mediate many of the intracellular actions of

insulin and other growth factors. Here we show that FKHR, a human homologue of the DAF16 transcription factor in Caenorhabditis elegans, is rapidly phosphorylated by human protein kinase Balpha (**PKBalpha**) at Thr-24, Ser-256, and Ser-319 in vitro and at a much faster rate than BAD, which is thought to be a physiological substrate for PKB. The same three sites, which all lie in the canonical PKB consensus sequences (Arg-Xaa-Arg-Xaa-Xaa-(Ser/Thr)), became phosphorylated when FKHR was cotransfected with either PKB or **PDK1** (an upstream activator of PKB). All three residues became phosphorylated when 293 cells were stimulated with insulin-like growth factor 1 (IGF-1). The IGF-1-induced phosphorylation was abolished by the PtdIns 3-kinase inhibitor wortmannin but not by PD 98059 (an inhibitor of the mitogen-activated protein kinase cascade) or by rapamycin. These results indicate that FKHR is a physiological substrate of PKB and that it may mediate some of the physiological effects of PKB on gene expression. DAF16 is known to be a component of a signaling pathway that has been partially dissected genetically and includes homologues of the insulin/IGF-1 receptor, PtdIns 3-kinase and PKB. The conservation of Thr-24, Ser-256, and Ser-319 and the sequences surrounding them in DAF16 therefore suggests that DAF16 is also a direct substrate for PKB in C. elegans.

L10 ANSWER 14 OF 19 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1999112925 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9895304
TITLE: Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-**phosphoinositide**-dependent protein kinase-1.
AUTHOR: Currie R A; Walker K S; Gray A; Deak M; Casamayor A; Downes C P; Cohen P; Alessi D R; Lucocq J
CORPORATE SOURCE: Department of Biochemistry, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, U.K.. racurrie@bad.dundee.ac.uk
SOURCE: Biochemical journal, (1999 Feb 1) 337 (Pt 3) 575-83. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990413
Last Updated on STN: 20020420
Entered Medline: 19990330

AB 3-**Phosphoinositide**-dependent protein kinase-1 (**PDK1**) interacts stereoselectively with the d-enantiomer of PtdIns(3,4,5)P3 (KD 1.6 nM) and PtdIns(3,4)P2 (KD 5.2 nM), but binds with lower affinity to PtdIns3P or PtdIns(4,5)P2. The binding of PtdIns(3,4,5)P3 to **PDK1** was greatly decreased by making specific mutations in the pleckstrin homology (PH) domain of **PDK1** or by deleting it. The same mutations also greatly decreased the rate at which **PDK1** activated protein kinase Balpha (**PKBalpha**) in vitro in the presence of lipid vesicles containing PtdIns(3,4,5)P3, but did not affect the rate at which **PDK1** activated a **PKBalpha** mutant lacking the PH domain in the absence of PtdIns(3,4,5)P3. When overexpressed in 293 or PAE cells, **PDK1** was located at the plasma membrane and in the cytosol, but was excluded from the nucleus. Mutations that disrupted the interaction of PtdIns(3,4,5)P3 or PtdIns(4,5)P2 with **PDK1** abolished the association of **PDK1** with the plasma membrane. Growth-factor stimulation promoted the translocation of transfected **PKBalpha** to the plasma membrane, but had no effect on the subcellular distribution of **PDK1** as judged by immunoelectron microscopy of fixed cells. This conclusion was also supported by confocal microscopy of green fluorescent protein-**PDK1** in live cells. These results, together with

previous observations, indicate that PtdIns(3,4,5)P3 plays several roles in the **PDK1**-induced activation of **PKBalpha**. First, it binds to the PH domain of PKB, altering its conformation so that it can be activated by **PDK1**. Secondly, interaction with PtdIns(3,4,5)P3 recruits PKB to the plasma membrane with which **PDK1** is localized constitutively by virtue of its much stronger interaction with PtdIns(3,4,5)P3 or PtdIns(4,5)P2. Thirdly, the interaction of **PDK1** with PtdIns(3,4,5)P3 facilitates the rate at which it can activate PKB.

L10 ANSWER 15 OF 19 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1999244939 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10226025
TITLE: **PDK1** acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2.
AUTHOR: Balendran A; Casamayor A; Deak M; Paterson A; Gaffney P; Currie R; Downes C P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK.
SOURCE: Current biology : CB, (1999 Apr 22) 9 (8) 393-404. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20020420
Entered Medline: 19990601
AB BACKGROUND: Protein kinase B (PKB) is activated by phosphorylation of Thr308 and of Ser473. Thr308 is phosphorylated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) but the identity of the kinase that phosphorylates Ser473 (provisionally termed PDK2) is unknown. RESULTS: The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (PRK2), termed the **PDK1**-interacting fragment (PIF). PIF is situated carboxy-terminal to the kinase domain of PRK2, and contains a consensus motif for phosphorylation by PDK2 similar to that found in **PKBalpha**, except that the residue equivalent to Ser473 is aspartic acid. Mutation of any of the conserved residues in the PDK2 motif of PIF prevented interaction of PIF with **PDK1**. Remarkably, interaction of **PDK1** with PIF, or with a synthetic peptide encompassing the PDK2 consensus sequence of PIF, converted **PDK1** from an enzyme that could phosphorylate only Thr308 of **PKBalpha** to one that phosphorylates both Thr308 and Ser473 of **PKBalpha** in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3). Furthermore, the interaction of PIF with **PDK1** converted the **PDK1** from a form that is not directly activated by PtdIns(3,4,5)P3 to a form that is activated threefold by PtdIns(3,4,5)P3. We have partially purified a kinase from brain extract that phosphorylates Ser473 of **PKBalpha** in a PtdIns(3,4,5)P3-dependent manner and that is immunoprecipitated with **PDK1** antibodies. CONCLUSIONS: **PDK1** and PDK2 might be the same enzyme, the substrate specificity and activity of **PDK1** being regulated through its interaction with another protein(s). PRK2 is a probable substrate for **PDK1**.

L10 ANSWER 16 OF 19 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1999175477 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10074427
TITLE: Functional counterparts of mammalian protein kinases **PDK1** and SGK in budding yeast.
AUTHOR: Casamayor A; Torrance P D; Kobayashi T; Thorner J; Alessi D

R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit Department of Biochemistry University of Dundee Dundee DD1 5EH Scotland UK.
CONTRACT NUMBER: GM21841 (NIGMS)
SOURCE: Current biology : CB, (1999 Feb 25) 9 (4) 186-97. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 20020420
Entered Medline: 19990422

AB BACKGROUND: In animal cells, recruitment of phosphatidylinositol 3-kinase by growth factor receptors generates 3-**phosphoinositides**, which stimulate 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**). Activated **PDK1** then phosphorylates and activates downstream protein kinases, including protein kinase B (PKB)/c-Akt, p70 S6 kinase, PKC isoforms, and serum- and glucocorticoid-inducible kinase (SGK), thereby eliciting physiological responses. RESULTS: We found that two previously uncharacterised genes of Saccharomyces cerevisiae, which we term PKH1 and PKH2, encode protein kinases with catalytic domains closely resembling those of human and Drosophila **PDK1**. Both Pkh1 and Pkh2 were essential for cell viability. Expression of human **PDK1** in otherwise inviable pkh1Delta pkh2Delta cells permitted growth. In addition, the yeast YPK1 and YKR2 genes were found to encode protein kinases each with a catalytic domain closely resembling that of SGK; both Ypk1 and Ykr2 were also essential for viability. Otherwise inviable ypk1Delta ykr2Delta cells were fully rescued by expression of rat SGK, but not mouse PKB or rat p70 S6 kinase. Purified Pkh1 activated mammalian SGK and **PKBalpha** in vitro by phosphorylating the same residue as **PDK1**. Pkh1 activated purified Ypk1 by phosphorylating the equivalent residue (Thr504) and was required for maximal Ypk1 phosphorylation in vivo. Unlike PKB, activation of Ypk1 and SGK by Pkh1 did not require phosphatidylinositol 3,4,5-trisphosphate, consistent with the absence of pleckstrin homology domains in these proteins. The phosphorylation consensus sequence for Ypk1 was similar to that for **PKBalpha** and SGK. CONCLUSIONS: Pkh1 and Pkh2 function similarly to **PDK1**, and Ypk1 and Ykr2 to SGK. As in animal cells, these two groups of yeast kinases constitute two tiers of a signalling cascade required for yeast cell growth.

L10 ANSWER 17 OF 19 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1998180962 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9512493
TITLE: Activation of protein kinase B beta and gamma isoforms by insulin in vivo and by 3-**phosphoinositide** -dependent protein kinase-1 in vitro: comparison with protein kinase B alpha.
AUTHOR: Walker K S; Deak M; Paterson A; Hudson K; Cohen P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.. kswalker@BAD.dundee.ac.uk
SOURCE: Biochemical journal, (1998 Apr 1) 331 (Pt 1) 299-308. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520

Last Updated on STN: 20020420
Entered Medline: 19980513

AB The regulatory and catalytic properties of the three mammalian isoforms of protein kinase B (PKB) have been compared. All three isoforms (**PKBalpha**, PKBbeta and PKBgamma) were phosphorylated at similar rates and activated to similar extents by 3-**phosphoinositide** -dependent protein kinase-1 (**PDK1**). Phosphorylation and activation of each enzyme required the presence of PtdIns(3,4,5)P3 or PtdIns(3,4)P2, as well as **PDK1**. The activation of PKBbeta and PKBgamma by **PDK1** was accompanied by the phosphorylation of the residues equivalent to Thr308 in **PKBalpha**, namely Thr309 (PKBbeta) and Thr305 (PKBgamma). PKBgamma which had been activated by **PDK1** possessed a substrate specificity identical with that of **PKBalpha** and PKBbeta towards a range of peptides. The activation of PKBgamma and its phosphorylation at Thr305 was triggered by insulin-like growth factor-1 in 293 cells. Stimulation of rat adipocytes or rat hepatocytes with insulin induced the activation of **PKBalpha** and PKBbeta with similar kinetics. After stimulation of adipocytes, the activity of PKBbeta was twice that of **PKBalpha**, but in hepatocytes **PKBalpha** activity was four-fold higher than PKBbeta. Insulin induced the activation of **PKBalpha** in rat skeletal muscle in vivo, with little activation of PKBbeta. Insulin did not induce PKBgamma activity in adipocytes, hepatocytes or skeletal muscle, but PKBgamma was the major isoform activated by insulin in rat L6 myotubes (a skeletal-muscle cell line).

L10 ANSWER 18 OF 19 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:4391 BIOSIS
DOCUMENT NUMBER: PREV199800004391
TITLE: Further evidence that the inhibition of glycogen synthase kinase-3beta by IGF-1 is mediated by **PDK1** /PKB-induced phosphorylation of Ser-9 and not by dephosphorylation of Tyr-216.
AUTHOR(S): Shaw, Morag [Reprint author]; Cohen, Philip; Alessi, Dario R.
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Dep. Biochem., Univ. Dundee, Dundee DD1 4HN, UK
SOURCE: FEBS Letters, (Oct. 27, 1997) Vol. 416, No. 3, pp. 307-311. print.
CODEN: FEBLAL. ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Dec 1997
Last Updated on STN: 24 Feb 1998

AB 293 cells were transfected with wild-type GSK3beta (WT-GSK3beta) or a mutant in which the PKB phosphorylation site (Ser-9) was altered to Ala (A9-GSK3beta). Upon stimulation with IGF-1 or insulin, WT-GSK3beta was inhibited 75% or 60%, respectively, whereas the activity of the A9-GSK3beta mutant was unaffected. Incubation of WT-GSK3beta with PP2A, (a Ser/Thr-specific phosphatase) completely reversed the IGF-1- or insulin-induced inhibition. IGF-1 stimulation did not induce any tyrosine dephosphorylation of WT-GSK3beta or A9-GSK3beta. Coexpression of WT-GSK3beta in 293 cells with either **PKBalpha** (also known as AKT) or **PDK1** (the 'upstream' activator of PKB) mimicked the IGF-1- or insulin-induced phosphorylation of Ser-9 and inactivation of GSK3beta.

L10 ANSWER 19 OF 19 MEDLINE on STN

ACCESSION NUMBER: 97250749 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9094314
TITLE: Characterization of a 3-**phosphoinositide** -dependent protein kinase which phosphorylates and

activates protein kinase Balpha.

AUTHOR: Alessi D R; James S R; Downes C P; Holmes A B; Gaffney P R; Reese C B; Cohen P

CORPORATE SOURCE: Medical Research Council Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee, DD1 4HN, Scotland.. dralessi@bad.dundee.ac.uk

SOURCE: Current biology : CB, (1997 Apr 1) 7 (4) 261-9. Journal code: 9107782. ISSN: 0960-9822.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF017995

ENTRY MONTH: 199705

ENTRY DATE: Entered STN: 19970609
Last Updated on STN: 20020420
Entered Medline: 19970529

AB BACKGROUND: Protein kinase B (PKB), also known as c-Akt, is activated rapidly when mammalian cells are stimulated with insulin and growth factors, and much of the current interest in this enzyme stems from the observation that it lies 'downstream' of **phosphoinositide** 3-kinase on intracellular signalling pathways. We recently showed that insulin or insulin-like growth factor 1 induce the phosphorylation of PKB at two residues, Thr308 and Ser473. The phosphorylation of both residues is required for maximal activation of PKB. The kinases that phosphorylate PKB are, however, unknown. RESULTS: We have purified 500 000-fold from rabbit skeletal muscle extracts a protein kinase which phosphorylates **PKBalpha** at Thr308 and increases its activity over 30-fold. We tested the kinase in the presence of several inositol phospholipids and found that only low micromolar concentrations of the D enantiomers of either phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P3) or PtdIns(3,4)P2 were effective in potently activating the kinase, which has been named PtdIns(3,4,5)P3-dependent protein kinase-1 (**PDK1**). None of the inositol phospholipids tested activated or inhibited **PKBalpha** or induced its phosphorylation under the conditions used. **PDK1** activity was not affected by wortmannin, indicating that it is not likely to be a member of the **phosphoinositide** 3-kinase family. CONLCUSIONS: **PDK1** is likely to be one of the protein kinases that mediate the activation of PKB by insulin and growth factors. **PDK1** may, therefore, play a key role in mediating many of the actions of the second messenger(s) PtdIns(3,4, 5)P3 and/or PtdIns(3,4)P2.

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=> d his

     (FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005
L1          1799 S "PDK1"
L2         62622 S PHOSPHOINOSITIDE##
L3          1051 S L1 AND L2
L4          2934 S  "PIF" OR "PRK2"
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7
L9            35 S L3 AND PKBALPHA
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)

=> s l3 and "PDK2"
L11           67 L3 AND "PDK2"
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=> dup rem l1
<------User Break------>

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12          24 DUP REM L11 (43 DUPLICATES REMOVED)

=> d 1-24 ibib ab

L12  ANSWER 1 OF 24     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2004586613     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 15364915
TITLE:               Protein kinase C betaII regulates Akt phosphorylation on
                     Ser-473 in a cell type- and stimulus-specific fashion.
COMMENT:             Erratum in: J Biol Chemical 2005 Mar 4;280(9):8628
AUTHOR:              Kawakami Yuko; Nishimoto Hajime; Kitaura Jiro;
                     Maeda-Yamamoto Mari; Kato Roberta M; Littman Dan R; Leitges
                     Michael; Rawlings David J; Kawakami Toshiaki
CORPORATE SOURCE:    Division of Cell Biology, La Jolla Institute for Allergy
                     and Immunology, San Diego, California 92121, USA.
CONTRACT NUMBER:     AI33617 (NIAID)
     AI38348 (NIAID)
SOURCE:              Journal of biological chemistry, (2004 Nov 12) 279 (46)
                     47720-5.  Electronic Publication: 2004-09-09.
                     Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200501
ENTRY DATE:          Entered STN: 20041125
                     Last Updated on STN: 20050122
                     Entered Medline: 20050121
AB   Akt (= protein kinase B), a subfamily of the AGC serine/threonine kinases,
     plays critical roles in survival, proliferation, glucose metabolism, and
     other cellular functions.  Akt activation requires the recruitment of the
     enzyme to the plasma membrane by interacting with membrane-bound lipid
     products of phosphatidylinositol 3-kinase.  Membrane-bound Akt is then
     phosphorylated at two sites for its full activation; Thr-308 in the
     activation loop of the kinase domain is phosphorylated by 3-
     phosphoinositide-dependent kinase-1 (PDK1) and Ser-473
     in the C-terminal hydrophobic motif by a putative kinase PDK2.
     The identity of PDK2 has been elusive.  Here we present evidence
     that conventional isoforms of protein kinase C (PKC), particularly
     PKCbetaII, can regulate Akt activity by directly phosphorylating Ser-473
     in vitro and in IgE/antigen-stimulated mast cells.  By contrast, PKCbeta
     is not required for Ser-473 phosphorylation in mast cells stimulated with
     stem cell factor or interleukin-3, in serum-stimulated fibroblasts, or in
     antigen receptor-stimulated T or B lymphocytes.  Therefore, PKCbetaII
     appears to work as a cell type- and stimulus-specific PDK2.

L12  ANSWER 2 OF 24  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation  on
     STN
ACCESSION NUMBER:    2004:841351  SCISEARCH
THE GENUINE ARTICLE: 854PW
TITLE:               Identification of a PKB/Akt hydrophobic motif Ser-473
                     kinase as DNA-dependent protein kinase
AUTHOR:              Feng J H; Park J; Cron P; Hess D; Hemmings B A (Reprint)
CORPORATE SOURCE:    Friedrich Miescher Inst Biomed Res, Maulbeerstr 66,
                     CH-4058 Basel, Switzerland (Reprint); Friedrich Miescher
                     Inst Biomed Res, CH-4058 Basel, Switzerland
                     brian.hemmings@fmi.ch
COUNTRY OF AUTHOR:   Switzerland
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SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (24 SEP 2004) Vol. 279, No. 39, pp. 41189-41196.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 70
ENTRY DATE: Entered STN: 15 Oct 2004
Last Updated on STN: 15 Oct 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Full activation of protein kinase B (PKB)/Akt requires phosphorylation on Thr-308 and Ser-473 by 3-**phosphoinositide**-dependent kinase-1 (**PDK1**) and Ser-473 kinase (S473K), respectively. Although **PDK1** has been well characterized, the identification of the S473K remains controversial. A major PKB Ser-473 kinase activity was purified from the membrane fraction of HEK293 cells and found to be DNA-dependent protein kinase (DNA-PK). DNA-PK co-localized and associated with PKB at the plasma membrane. In vitro, DNA-PK phosphorylated PKB on Ser-473, resulting in a similar to10-fold enhancement of PKB activity. Knockdown of DNA-PK by small interfering RNA inhibited Ser-473 phosphorylation induced by insulin and pervanadate. DNA-PK-deficient glioblastoma cells did not respond to insulin at the level of Ser-473 phosphorylation; this effect was restored by complementation with the human PRKDC gene. We conclude that DNA-PK is a long sought after kinase responsible for the Ser-473 phosphorylation step in the activation of PKB.

L12 ANSWER 3 OF 24 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2004501625 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15470109
TITLE: Differential roles of **PDK1**- and **PDK2**-phosphorylation sites in the yeast AGC kinases Ypk1, Pkc1 and Sch9.
AUTHOR: Roelants Francoise M; Torrance Pamela D; Thorner Jeremy
CORPORATE SOURCE: Department of Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720-3202, USA.
CONTRACT NUMBER: CA09041 (NCI)
GM07232 (NIGMS)
GM21841 (NIGMS)
SOURCE: Microbiology (Reading, England), (2004 Oct) 150 (Pt 10) 3289-304.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200501
ENTRY DATE: Entered STN: 20041008
Last Updated on STN: 20050114
Entered Medline: 20050113

AB Saccharomyces cerevisiae Pkh1 and Pkh2 (orthologues of mammalian protein kinase, **PDK1**) are functionally redundant. These kinases activate three AGC family kinases involved in the maintenance of cell wall integrity: Ypk1 and Ypk2, two closely related, functionally redundant enzymes (orthologues of mammalian protein kinase SGK), and Pkc1 (orthologue of mammalian protein kinase PRK2). Pkh1 and Pkh2 activate Ypk1, Ypk2 and Pkc1 by phosphorylating a Thr in a conserved sequence motif (**PDK1** site) within the activation loop of these proteins. A fourth protein kinase involved in growth control and stress response, Sch9 (orthologue of mammalian protein kinase c-Akt/PKB), also carries the conserved activation loop motif. Like other AGC family kinases, Ypk1, Ypk2, Pkc1 and Sch9 also carry a second conserved sequence motif situated

in a region C-terminal to the catalytic domain, called the hydrophobic motif (**PDK2** site). Currently, there is still controversy surrounding the identity of the enzyme responsible for phosphorylating this second site and the necessity for phosphorylation at this site for in vivo function. Here, genetic and biochemical methods have been used to investigate the physiological consequences of phosphorylation at the **PDK1** and **PDK2** sites of Ypk1, Pkc1 and Sch9. It was found that phosphorylation at the **PDK1** site in the activation loop is indispensable for the essential functions of all three kinases in vivo, whereas phosphorylation at the **PDK2** motif plays a non-essential and much more subtle role in modulating the ability of these kinases to regulate the downstream processes in which they participate.

L12 ANSWER 4 OF 24 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2004259252 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15157674
TITLE: Regulation of protein kinase B/Akt activity and Ser473 phosphorylation by protein kinase Calpha in endothelial cells.
AUTHOR: Partovian Chohreh; Simons Michael
CORPORATE SOURCE: Department of Medicine, Angiogenesis Research Center and Section of Cardiology, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756, USA.
CONTRACT NUMBER: HL62289 (NHLBI)
HL63609 (NHLBI)
SOURCE: Cellular signalling, (2004 Aug) 16 (8) 951-7.
Journal code: 8904683. ISSN: 0898-6568.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200505
ENTRY DATE: Entered STN: 20040526
Last Updated on STN: 20050520
Entered Medline: 20050519

AB Protein kinase Balpha (PKBalpha/Akt-1) is a key mediator of multiple signaling pathways involved in angiogenesis, cell proliferation and apoptosis among others. The unphosphorylated form of Akt-1 is virtually inactive and its full activation requires two phosphatidylinositol-3,4,5-triphosphate-dependent phosphorylation events, Thr308 by 3-**phosphoinositide**-dependent kinase-1 (**PDK1**) and Ser473 by an undefined kinase that has been termed **PDK2**. Recent studies have suggested that the Ser473 kinase is a plasma membrane raft-associated kinase. In this study we show that protein kinase Calpha (PKCalpha) translocates to the membrane rafts in response to insulin growth factor-1 (IGF-1) stimulation. Overexpression of PKCalpha increases Ser473 phosphorylation and Akt-1 activity, while inhibition of its activity or expression decreases IGF-1-dependent activation of Akt-1. Furthermore, in vitro, in the presence of phospholipids and calcium, PKCalpha directly phosphorylates Akt-1 at the Ser473 site. We conclude, therefore, that PKCalpha regulates Akt-1 activity via Ser473 phosphorylation and may function as **PDK2** in endothelial cells.

L12 ANSWER 5 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:47158 BIOSIS
DOCUMENT NUMBER: PREV200500047938
TITLE: PI3K-Akt pathway: Its functions and alterations in human cancer.
AUTHOR(S): Osaki, M. [Reprint Author]; Oshimura, M.; Ito, H.
CORPORATE SOURCE: Div Organ PatholDept Microbiol and PatholFac Med, Tottori Univ, 86 Nishi Chi, Tottori, 6838503, Japan
osamitsu@grape.med.tottori-u.ac.jp

SOURCE: Apoptosis, (November 2004) Vol. 9, No. 6, pp. 667-676. print.
ISSN: 1360-8185 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Jan 2005
Last Updated on STN: 26 Jan 2005

AB Phosphatidylinositol-3-kinase (PI3K) is a lipid kinase and generates phosphatidylinositol-3,4,5-trisphosphate (PI(3, 4, 5) P3). PI(3, 4, 5) P3 is a second messenger essential for the translocation of Akt to the plasma membrane where it is phosphorylated and activated by **phosphoinositide**-dependent kinase (PDK) 1 and **PDK2**. Activation of Akt plays a pivotal role in fundamental cellular functions such as cell proliferation and survival by phosphorylating a variety of substrates. In recent years, it has been reported that alterations to the PI3K-Akt signaling pathway are frequent in human cancer. Constitutive activation of the PI3K-Akt pathway occurs due to amplification of the PIK3C gene encoding PI3K or the Akt gene, or as a result of mutations in components of the pathway, for example PTEN (phosphatase and tensin homologue deleted on chromosome 10), which inhibit the activation of Akt. Several small molecules designed to specifically target PI3K-Akt have been developed, and induced cell cycle arrest or apoptosis in human cancer cells in vitro and in vivo. Moreover, the combination of an inhibitor with various cytotoxic agents enhances the anti-tumor efficacy. Therefore, specific inhibition of the activation of Akt may be a valid approach to treating human malignancies and overcoming the resistance of cancer cells to radiation or chemotherapy.

L12 ANSWER 6 OF 24 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2004608584 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15581868
TITLE: **PDK1** is required for the hormonal signaling pathway leading to meiotic resumption in starfish oocytes.
AUTHOR: Hiraoka Daisaku; Hori-Oshima Sawako; Fukuhara Takeshi; Tachibana Kazunori; Okumura Eiichi; Kishimoto Takeo
CORPORATE SOURCE: Laboratory of Cell and Developmental Biology, Graduate School of Bioscience, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226-8501, Japan.
SOURCE: Developmental biology, (2004 Dec 15) 276 (2) 330-6.
Journal code: 0372762. ISSN: 0012-1606.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB110536
ENTRY MONTH: 200503
ENTRY DATE: Entered STN: 20041208
Last Updated on STN: 20050401
Entered Medline: 20050331

AB Meiotic resumption is generally under the control of an extracellular maturation-inducing hormone. It is equivalent to the G2-M phase transition in somatic cell mitosis and is regulated by cyclin B-Cdc2 kinase. However, the complete signaling pathway from the hormone to cyclin B-Cdc2 is yet unclear in any organism. A model system to analyze meiotic resumption is the starfish oocyte, in which Akt/protein kinase B (PKB) plays a key mediator in hormonal signaling that leads to cyclin B-Cdc2 activation. Here we show in starfish oocytes that when **PDK1** activity is inhibited by a neutralizing antibody, maturation-inducing hormone fails to induce cyclin B-Cdc2 activation at the meiotic G2-M phase transition, even though **PDK2** activity becomes detectable. These observations assign a novel role to **PDK1** for a hormonal signaling intermediate toward meiotic resumption. They further support that **PDK2** is a molecule

distinct from **PDK1** and Akt, and that **PDK2** activity is not sufficient for the full activation of Akt in the absence of **PDK1** activity.

L12 ANSWER 7 OF 24 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2003268174 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12682057
TITLE: **Phosphoinositide**-dependent kinase-2 is a distinct protein kinase enriched in a novel cytoskeletal fraction associated with adipocyte plasma membranes.
AUTHOR: Hresko Richard C; Murata Haruhiko; Mueckler Mike
CORPORATE SOURCE: Department of Cell Biology and Physiology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA.
CONTRACT NUMBER: DK 38495 (NIDDK)
SOURCE: Journal of biological chemistry, (2003 Jun 13) 278 (24) 21615-22. Electronic Publication: 2003-04-07. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 20030610
Last Updated on STN: 20030723
Entered Medline: 20030722

AB By recombining subcellular components of 3T3-L1 adipocytes in a test tube, early insulin signaling events dependent on phosphatidylinositol 3-kinase (PI 3-kinase) were successfully reconstituted, up to and including the phosphorylation of glycogen synthase kinase-3 by the serine/threonine kinase, Akt (Murata, H., Hresko, R.C., and Mueckler, M. (2003) J. Biol. Chemical 278, 21607-21614). Utilizing the advantages provided by a cell-free methodology, we characterized **phosphoinositide**-dependent kinase 2 (**PDK2**), the putative kinase responsible for phosphorylating Akt on Ser-473. Immunodepleting cytosolic **PDK1** from an in vitro reaction containing plasma membrane and cytosol markedly inhibited insulin-stimulated phosphorylation of Akt at the **PDK1** site (Thr-308) but had no effect on phosphorylation at the **PDK2** site (Ser-473). In contrast, **PDK2** activity was found to be highly enriched in a novel cytoskeletal subcellular fraction associated with plasma membranes. Akt isoforms 1-3 and a kinase-dead Akt1 (K179A) mutant were phosphorylated in a phosphatidylinositol 3,4,5-trisphosphate-dependent manner at Ser-473 in an in vitro reaction containing this novel adipocyte subcellular fraction. Our data indicate that this **PDK2** activity is the result of a kinase distinct from **PDK1** and is not due to autophosphorylation or transphosphorylation of Akt.

L12 ANSWER 8 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:204833 BIOSIS
DOCUMENT NUMBER: PREV200400205373
TITLE: The effect of Akt by antidepressants in the rat brain.
AUTHOR(S): Misonoo, A. [Reprint Author]; Kenichi, O. [Reprint Author]; Hsagawa, H. [Reprint Author]; Kiyofumi, T. [Reprint Author]; Kanai, S. [Reprint Author]; Tanaka, D. [Reprint Author]; Hisinuma, T. [Reprint Author]; Fujii, S. [Reprint Author]; Sasuga, Y. [Reprint Author]; Miyamoto, S. [Reprint Author]; Asakura, M. [Reprint Author]
CORPORATE SOURCE: Dept. Neuropsych, St. Marianna Univ. Sch. Med, Kawasaki, Japan
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 849.15. http://sfn.scholarone.com. e-file.

Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Apr 2004
Last Updated on STN: 14 Apr 2004

AB Akt, also known as protein kinase B, is a protein kinase as a downstream kinase of **phosphoinositide** 3-kinase (PI3-K) and BDNF. Phoshporylation of residues Ser-473 and Thr-308 is required for Akt activity by **PDK1** and **PDK2**, respectively. PRK2 inhibits the phosphorylation of Akt Ser-473 by **PDK1**. Key roles for Akt in cellular processes such as apotosis, neurotransmitters release and transcription are now well established.The phosphorylation of Akt Ser-473 and Thr-308 increased after 3 weeks Clomipramine and Fluvoxamine treatment by Immunoblot measurement. **PDK1** and **PDK1**, Ser-241 phosphorylation also increased after treatment of antidepressants. But PI3-K and PRK2 were not changed by antidepressants.Akt is known to play a role in the releasing process for several neurotransmitters (5-HT and NE). It is important cellular mechanism for antidepressants that Akt activated by PDK.

L12 ANSWER 9 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:50819 BIOSIS
DOCUMENT NUMBER: PREV200400051207
TITLE: **PDK2** activity of protein kinase Calpha: Regulation of protein kinase B/Akt activity in endothelial cells.
AUTHOR(S): Partovian, Chohreh [Reprint Author]; Simons, Michael [Reprint Author]
CORPORATE SOURCE: Dartmouth Med Sch, Lebanon, NH, USA
SOURCE: Circulation, (October 28 2003) Vol. 108, No. 17 Supplement, pp. IV-3. print.
Meeting Info.: American Heart Association Scientific Sessions 2003. Orlando, FL, USA. November 09-12, 2003. American Heart Association.
ISSN: 0009-7322 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Jan 2004
Last Updated on STN: 21 Jan 2004

L12 ANSWER 10 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:258182 BIOSIS
DOCUMENT NUMBER: PREV200300258182
TITLE: Regulation of epithelial-mesenchymal transformation in palate development.
AUTHOR(S): Kang, Pei [Reprint Author]; Svoboda, Kathy K H
CORPORATE SOURCE: Baylor College of Dentistry, Texas AandM University Health Science Center, 3302 Gaston Ave, Dallas, TX, 75246, USA pkang@tambcd.edu; ksvoboda@tambcd.edu
SOURCE: FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 718.5. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB.
ISSN: 0892-6638 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English

ENTRY DATE: Entered STN: 4 Jun 2003
Last Updated on STN: 4 Jun 2003

AB During development, palatal shelf medial edge epithelia (MEE) make contact, adhere and form desmosomes. The epithelia thin to one layer, lose cell-cell adhesion, degrade the basal lamina, become fusiform and migrate into mesenchyme. This is termed epithelial-mesenchymal transformation (EMT). TGF[beta]3 and PI-3 kinase are essential for EMT. Alternatively, high doses of nicotine or its receptor antagonists block EMT and palatal fusion in vitro. TGF[beta]3 can signal through the Smad or the PI-3 kinase pathway. The nicotinic acetylcholine receptors (nAChRs) also signal through the PI-3 kinase pathway. Activiated PI-3 kinase stimulates **PDK1**/2 (integrin-linked kinase) that phosphorylates PKB/Akt, at serine 473. In this study the activity of PI-3 kinase was investigated by assaying the activation of Akt (Ser473). Palatal shelves from 13.5 day mouse embryos were cultured for 20 hrs in serum free media with or without 6 mM nicotine or LY294002 (1[mu]M, and 10[mu]M), a PI-3 kinase inhibitor. Akt(ser473) was immuno-localized on cryostat sections. Western blots of MEE isolated from whole cultured palates determined the amount of active Akt. Phosphorylated Akt was decreased in palates exposed to nicotine in western blots and immunolocalization studies. In conclusion, blocking PI-3 kinase or treating with nicotine decreased the activity of Akt. These observations provide evidence that the PI-3 kinase pathway may cross talk with nAChR signaling for EMT during palate fusion.

L12 ANSWER 11 OF 24 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2002408883 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12162751
TITLE: Characterization of **PDK2** activity against protein kinase B gamma.
AUTHOR: Hodgkinson Conrad P; Sale Elizabeth M; Sale Graham J
CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Southampton SO16 7PX, United Kingdom.
SOURCE: Biochemistry, (2002 Aug 13) 41 (32) 10351-9.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020807
Last Updated on STN: 20020904
Entered Medline: 20020903

AB Protein kinase B (PKB), also known as Akt, is a serine/threonine protein kinase controlled by insulin, various growth factors, and phosphatidylinositol 3-kinase. Full activation of the PKB enzyme requires phosphorylation of a threonine in the activation loop and a serine in the C-terminal tail. **PDK1** has clearly been shown to phosphorylate the threonine, but the mechanism leading to phosphorylation of the serine, the **PDK2** site, is unclear. A yeast two-hybrid screen using full-length human PKBgamma identified protein kinase C (PKC) zeta, an atypical PKC, as an interactor with PKBgamma, an association requiring the pleckstrin homology domain of PKBgamma. Endogenous PKBgamma was shown to associate with endogenous PKCzeta both in cos-1 cells and in 3T3-L1 adipocytes, demonstrating a physiological interaction. Immunoprecipitates of PKCzeta, whether endogenous PKCzeta from insulin-stimulated 3T3-L1 adipocytes or overexpressed PKCzeta from cos-1 cells, phosphorylated S472 (the C-terminal serine phosphorylation site) of PKBgamma, in vitro. In vivo, overexpression of PKCzeta stimulated the phosphorylation of approximately 50% of the PKBgamma molecules, suggesting a physiologically meaningful effect. However, pure PKCzeta protein was incapable of phosphorylating S472 of PKBgamma. Antisense knockout studies and use of a

PDK1 inhibitor showed that neither PKB autophosphorylation nor phosphorylation by **PDK1** accounted for the S472 phosphorylation in PKCzeta immunoprecipitates. Staurosporine inhibited the PKCzeta activity but not the **PDK2** activity in PKCzeta immunoprecipitates. Together these results indicate that an independent **PDK2** activity exists that physically associates with PKCzeta and that PKCzeta, by binding PKBgamma, functions to deliver the **PDK2** to a required location. PKCzeta thus functions as an adaptor, associating with a staurosporine-insensitive **PDK2** enzyme that catalyzes the phosphorylation of S472 of PKBgamma. Because both PKCzeta and PKB have been proposed to be required for mediating a number of crucial insulin responses, formation of an active signaling complex containing PKCzeta, PKB, and **PDK2** is an attractive mechanism for ensuring that all the critical sites on targets such as glycogen synthase kinase-3 are phosphorylated.

L12 ANSWER 12 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:459042 SCISEARCH
THE GENUINE ARTICLE: 555NX
TITLE: Activation of SGK1 by HGF, Rac1 and integrin-mediated cell adhesion in MDCK cells: PI-3K-dependent and -independent pathways
AUTHOR: Shelly C; Herrera R (Reprint)
CORPORATE SOURCE: Pfizer Co, Ann Arbor Labs, Dept Cell Biol Global Res & Dev, Ann Arbor, MI 48105 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF CELL SCIENCE, (1 MAY 2002) Vol. 115, No. 9, pp. 1985-1993.
ISSN: 0021-9533.
PUBLISHER: COMPANY OF BIOLOGISTS LTD, BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 54
ENTRY DATE: Entered STN: 14 Jun 2002
Last Updated on STN: 14 Jun 2002

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The SGK1 protein belongs to the AGC gene family of kinases that are regulated by phosphorylation mediated by **PDK1**. SGK1 regulation is accomplished by several pathways including growth-factor and stress-mediated signaling. We have expanded the analysis of SGK1 regulation in epithelial cells. We used HA-tagged SGK1 to transiently transfect MDCK cells and study the regulation of SGK1 upon stimulation with HGF, cAMP or upon adhesion of the cells to immobilized fibronectin. In addition, we studied the regulation of SGK1 activity by small GTP-binding proteins of the Rho family.

Treatment of MDCK cells with HGF leads to a time-dependent activation of SGK1 that is blocked by wortmanin. This activation requires the conserved phosphorylation site present in the activation loop of the kinase (T256 in SGK1) and the phosphorylation site present in a hydrophobic domain at its C-terminus (S422 in SGK1), which are targets for **PDK1**/**PDK2**-mediated regulation of SGK1. We tested whether SGK1 could be activated by cAMP as it contains a putative PKA site. We were unable to demonstrate a significant activation of HA-SGK1 by cAMP stimulation under conditions where we detect cAMP-mediated phosphorylation of the transcription factor CREB.

Cotransfection of SGK1 with activated small GTP-binding proteins revealed that Rac1, but not Rho or Rap1, induces activation of SGK1. However, this activation was wortmanin insensitive and dominant-negative Rac1 did not inhibit the HGF-mediated activation of SGK1. Adhesion of MDCK cells to immobilized fibronectin also leads to activation of SGK1.

However, it appears that the integrin-mediated activation of HA-SGK1 differs from AKT activation in the fact that AKT phosphorylation was blocked by wortmanin (or LY294002) whereas HA-SGK1 was not. The adhesion-dependent activation, however, requires the intact phosphorylation sites of SGK1. Co-transfection of HA-SGK1 with RacV12 results in increased activity in adherent cells compared with HA-SGK1 alone. Since RacN17 failed to inhibit adhesion dependent-activation of SGK1, it suggests that integrin activation is achieved by a parallel Rae-independent pathway.

The activation of SGK1 by HGF and integrin provides a link between HGF-mediated protection of MDCK from deattachment induced apoptosis (anoikis). We demonstrate that dephosphorylation of the transcription factor FKRHL1 induced by cell de-attachment is prevented by activated SGK1, suggesting that SGK1 regulates cell survival pathways.

In summary, we demonstrate that SGK1 activation could be achieved through signaling pathways involved in the regulation of cell survival, cell-cell and cell-matrix interactions. SGK1 activation can be accomplished via HGF, PI-3K-dependent pathways and by integrin-mediated, PI-3K independent pathways. In addition, activation of SGK1 by the small GTP-binding protein Rac1 has been observed.

L12 ANSWER 13 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:393606 BIOSIS
DOCUMENT NUMBER: PREV200200393606
TITLE: Role of Akt signaling in vascular homeostasis and angiogenesis.
AUTHOR(S): Shiojima, Ichiro; Walsh, Kenneth [Reprint author]
CORPORATE SOURCE: Molecular Cardiology/CVI, Boston University School of Medicine, 715 Albany St, W611, Boston, MA, 02118, USA kwalsh@world.std.com
SOURCE: Circulation Research, (June 28, 2002) Vol. 90, No. 12, pp. 1243-1250. print.
CODEN: CIRUAL. ISSN: 0009-7330.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jul 2002
Last Updated on STN: 29 Aug 2002

AB Akt is a serine/threonine protein kinase that is activated by a number of growth factors and cytokines in a phosphatidylinositol-3 kinase-dependent manner. Although antiapoptotic activity of Akt is well known, it also regulates other aspects of cellular functions, including migration, glucose metabolism, and protein synthesis. In this review, Akt signaling in endothelial cells and its critical roles in the regulation of vascular homeostasis and angiogenesis will be discussed.

L12 ANSWER 14 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:167581 BIOSIS
DOCUMENT NUMBER: PREV200200167581
TITLE: Regulation of both **PDK1** and the phosphorylation of PKC-zeta and -delta by a C-terminal PRK2 fragment.
AUTHOR(S): Hodgkinson, Conrad P.; Sale, Graham J. [Reprint author]
CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Biomedical Sciences Building, Southampton, SO16 7PX, UK
G.J.Sale@soton.ac.uk
SOURCE: Biochemistry, (January 15, 2002) Vol. 41, No. 2, pp. 561-569. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article

LANGUAGE: English
ENTRY DATE: Entered STN: 5 Mar 2002
Last Updated on STN: 5 Mar 2002

AB The mechanism by which **PDK1** regulates AGC kinases remains unclear. To further understand this process, we performed a yeast two-hybrid screen using **PDK1** as bait. PKC-zeta, PKC-delta, and PRK2 were identified as interactors of **PDK1**. A combination of yeast two-hybrid binding assays and coprecipitation from mammalian cells was used to characterize the nature of the **PDK1**-PKC interaction. The presence of the PH domain of **PDK1** inhibited the interaction of **PDK1** with the PKCs. A contact region of **PDK1** was mapped between residues 314 and 408. The interaction of **PDK1** with the PKCs required the full-length PKC-zeta and -delta proteins apart from their C-terminal tails. **PDK1** was able to phosphorylate full-length PKC-zeta and -delta but not PKC-zeta and -delta constructs containing the **PDK1** phosphorylation site but lacking the C-terminal tails. A C-terminal PRK2 fragment, normally produced by caspase-3 cleavage during apoptosis, inhibited **PDK1** autophosphorylation by >90%. The ability of **PDK1** to phosphorylate PKC-zeta and -delta in vitro was also markedly inhibited by the PRK2 fragment. Additionally, generation of the PRK2 fragment in vivo inhibited by >90% the phosphorylation of endogenous PKC-zeta by **PDK1**. In conclusion, these results show that the C-terminal tail of PKC is a critical determinant for PKC-zeta and -delta phosphorylation by **PDK1**. Moreover, the C-terminal PRK2 fragment acts as a potent negative regulator of **PDK1** autophosphorylation and **PDK1** kinase activity against PKC-zeta and -delta. As the C-terminal PRK2 fragment is naturally generated during apoptosis, this may provide a mechanism of restraining prosurvival signals during apoptosis.

L12 ANSWER 15 OF 24 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2002494015 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12208782
TITLE: Gene expressions in Jurkat cells poisoned by a sulphur mustard vesicant and the induction of apoptosis.
AUTHOR: Zhang Peng; Ng Patrick; Caridha Diana; Leach Richard A; Asher Ludmila V; Novak Mark J; Smith William J; Zeichner Steven L; Chiang Peter K
CORPORATE SOURCE: Walter Reed Army Institute of Research, Silver Spring, Maryland, MD 20910-7500, USA.
SOURCE: British journal of pharmacology, (2002 Sep) 137 (2) 245-52. Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20021002
Last Updated on STN: 20030221
Entered Medline: 20030220

AB 1. The sulphur mustard vesicant 2-chloroethylethyl sulphide (CEES) induced apoptosis in Jurkat cells. 2. Akt (PKB), a pivotal protein kinase which can block apoptosis and promotes cell survival, was identified to be chiefly down-regulated in a dose-dependent manner following CEES treatment. Functional analysis showed that the attendant Akt activity was simultaneously reduced. 3. **PDK1**, an upstream effector of Akt, was also down-regulated following CEES exposure, but two other upstream effectors of Akt, PI3-K and **PDK2**, remained unchanged. 4. The phosphorylation of Akt at Ser(473) and Thr(308) was significantly decreased following CEES treatment, reflecting the suppressed kinase activity of both **PDK1** and **PDK2**. 5. Concurrently, the anti-apoptotic genes, Bcl family, were down-regulated, in sharp contrast to the striking up-regulation of some death executioner genes, caspase 3,

6, and 8. 6. Based on these findings, a model of CEES-induced apoptosis was established. These results suggest that CEES attacked the Akt pathway, directly or indirectly, by inhibiting Akt transcription, translation, and post-translation modification. 7. Taken together, upon exposure to CEES, apoptosis was induced in Jurkat cells via the down-regulation of the survival factors that normally prevent the activation of the death executioner genes, the caspases.

L12 ANSWER 16 OF 24 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2001269993 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11042204
TITLE: p38 Kinase-dependent MAPKAPK-2 activation functions as 3-**phosphoinositide**-dependent kinase-2 for Akt in human neutrophils.
AUTHOR: Rane M J; Coxon P Y; Powell D W; Webster R; Klein J B; Pierce W; Ping P; McLeish K R
CORPORATE SOURCE: Department of Medicine, University of Louisville Health Sciences Center and the Veterans Affairs Medical Center, Louisville, Kentucky 40202, USA.. mrane@louisville.edu
CONTRACT NUMBER: 1S10RR11368-01A1 (NCRR)
HL63901 (NHLBI)
SOURCE: Journal of biological chemistry, (2001 Feb 2) 276 (5) 3517-23. Electronic Publication: 2000-10-20.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20030105
Entered Medline: 20010621
AB Akt activation requires phosphorylation of Thr(308) and Ser(473) by 3-**phosphoinositide**-dependent kinase-1 and 2 (**PDK1** and **PDK2**), respectively. While **PDK1** has been cloned and sequenced, **PDK2** has yet to be identified. The present study shows that phosphatidylinositol 3-kinase-dependent p38 kinase activation regulates Akt phosphorylation and activity in human neutrophils. Inhibition of p38 kinase activity with SB203580 inhibited Akt Ser(473) phosphorylation following neutrophil stimulation with formyl-methionyl-leucyl-phenylalanine, FcgammaR cross-linking, or phosphatidylinositol 3,4,5-trisphosphate. Concentration inhibition studies showed that Ser(473) phosphorylation was inhibited by 0.3 microm SB203580, while inhibition of Thr(308) phosphorylation required 10 microm SB203580. Transient transfection of HEK293 cells with adenoviruses containing constitutively active MKK3 or MKK6 resulted in activation of both p38 kinase and Akt. Immunoprecipitation and glutathione S-transferase (GST) pull-down studies showed that Akt was associated with p38 kinase, MK2, and Hsp27 in neutrophils, and Hsp27 dissociated from the complex upon activation. Active recombinant MK2 phosphorylated recombinant Akt and Akt in anti-Akt, anti-MK2, anti-p38, and anti-Hsp27 immunoprecipitates, and this was inhibited by an MK2 inhibitory peptide. We conclude that Akt exists in a signaling complex containing p38 kinase, MK2, and Hsp27 and that p38-dependent MK2 activation functions as **PDK2** in human neutrophils.

L12 ANSWER 17 OF 24 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 2001517162 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11563975
TITLE: Tumour necrosis factor-alpha activation of protein kinase B in WEHI-164 cells is accompanied by increased phosphorylation of Ser473, but not Thr308.
AUTHOR: O'toole A; Moule S K; Lockyer P J; Halestrap A P

CORPORATE SOURCE: Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.
SOURCE: Biochemical journal, (2001 Oct 1) 359 (Pt 1) 119-27. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 20010924
Last Updated on STN: 20021211
Entered Medline: 20030807

AB Tumour necrosis factor-alpha (TNF-alpha) may activate both cell survival and cell death pathways. In the murine fibrosarcoma cell line WEHI-164, physiological concentrations (1 ng/ml) of TNF-alpha induced wortmannin-sensitive cell ruffling characteristic of the **phosphoinositide** 3-kinase (PI3-kinase) activation associated with cell survival. Wortmannin also enhanced cell death induced by TNF-alpha in the presence of actinomycin D, confirming that TNF-alpha activates a transcription-independent survival pathway requiring PI3-kinase activity. Both TNF-alpha and insulin-like growth factor 1 (IGF-1) caused a 6-10-fold wortmannin-sensitive increase in protein kinase B (PKB) activity within 5 min. For IGF-1, this was associated with an increase in phosphorylation of both Thr(308) and Ser(473), whereas for TNF-alpha only phosphorylation of Ser(473) was increased, even in the presence of okadaic acid to inhibit protein phosphatases 1 and 2A. TNF-alpha did not decrease the phosphorylation of Thr(308) induced by IGF-1, implying that TNF-alpha neither inhibits **phosphoinositide**-dependent kinase 1 (**PDK1**) nor activates an opposing phosphatase. In WEHI cells overexpressing a form of PKB, IGF-1 increased phosphorylation of Ser(473) on PKB, but not its kinase activity, whereas TNF-alpha failed to induce Ser(473) phosphorylation or kinase activation of either overexpressed T308A or wild-type PKB (where T308A is the mutant bearing the substitution Thr(308)-->A). IGF-1 caused translocation of green-fluorescent-protein-tagged ADP-ribosylation factor nucleotide-binding site opener (ARNO) to the plasma membrane of WEHI cells, but this was not detected with TNF-alpha. We conclude that, at physiological concentrations, TNF-alpha activates endogenous PKB by stimulating **PDK2** (increase in Ser(473) phosphorylation) in a PI3-kinase-dependent (wortmannin-sensitive) manner, without causing detectable stimulation of **PDK1** (no increase in Thr(308) phosphorylation) or ARNO translocation. Possible explanations of these observations are discussed.

L12 ANSWER 18 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2002004596 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11752635
TITLE: **PDK2**: a complex tail in one Akt.
AUTHOR: Chan T O; Tsichlis P N
CORPORATE SOURCE: The authors are at the Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA.. P_Tsichlis@lac.jci.tju.edu
CONTRACT NUMBER: RO1CA/GM80219 (NCI)
RO1CA38047 (NCI)
RO1CA56110 (NCI)
RO1CA57436 (NCI)
SOURCE: Science's STKE [electronic resource] : signal transduction knowledge environment, (2001 Jan 23) 2001 (66) PE1. Electronic Publication: 2001-01-23. Ref: 25
Journal code: 100964423. ISSN: 1525-8882.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020102
Last Updated on STN: 20020420
Entered Medline: 20020211

AB The kinase Akt contains two phosphatidylinositol-3 kinase (PI3K)-dependent phosphorylation sites, one in the activation loop (Thr(308)) and one in the carboxyl-terminal tail (Ser(473)), both of which are conserved among the members of the AGC kinase family. Under physiological conditions, the phosphorylation of Thr(308) appears to be coordinately regulated with the phosphorylation of Ser(473). Under experimental conditions, however, the two sites can be uncoupled, suggesting that their phosphorylation is controlled by different kinases and phosphatases. **Phosphoinositide**-dependent kinase 1 (**PDK1**), the kinase that phosphorylates the activation loop site, has been unambiguously identified. However, **PDK2**, a kinase that is hypothesized to phosphorylate the hydrophobic carboxyl-terminal site, remains elusive. This Perspective examines the regulation and biological significance of Akt phosphorylation at Ser(473). The authors propose that Ser(473) undergoes both autophosphorylation and phosphorylation by other kinases. Both events may be promoted by interactions between **PDK1** and phosphorylated or phosphomimetically altered hydrophobic phosphorylation motifs in kinases associated with Akt. These interactions may induce conformational changes in Akt that make Ser(473) accessible to phosphorylation.

L12 ANSWER 19 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:421165 HCAPLUS
DOCUMENT NUMBER: 133:68896
TITLE: Activating serum and glucocorticoid-induced protein kinase and drug screening
INVENTOR(S): Cohen, Philip; Kobayashi, Takayasu; Deak, Maria
PATENT ASSIGNEE(S): The University of Dundee, UK
SOURCE: PCT Int. Appl., 133 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2000035946	A1	20000622	WO 1999-GB4232		19991214
W: JP, US					
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
EP 1141003	A1	20011010	EP 1999-961205		19991214
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
JP 2002533063	T2	20021008	JP 2000-588203		19991214
PRIORITY APPLN. INFO.:			US 1998-112217P	P	19981214
			GB 1999-19676	A	19990819
			WO 1999-GB4232	W	19991214

AB A method of activating serum and glucocorticoid-induced protein kinase (SGK) is provided wherein the SGK is phosphorylated. The SGK may be phosphorylated by **PDK1** and/or a preparation containing **PDK2** activity. A method of identifying a compound that modulates the activity of SGK is provided, wherein the activity of SGK is measured by measuring the phosphorylation by SGK of a polypeptide comprising an amino acid sequence corresponding to the consensus sequence (Arg/Lys; preferably Arg)-X-(X/Arg)-X-X-(Ser/Thr)-Z wherein X indicates any amino acid, X/Arg indicates any amino acid, with a preference for arginine, and Z indicates

that the amino acid residue is preferably a hydrophobic residue. The SGK may be activated by phosphorylation. The invention relates to screening methods for finding new drugs or lead compds.
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 20 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:668345 SCISEARCH
THE GENUINE ARTICLE: 348GX
TITLE: The role of 3-**phosphoinositide**-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells
AUTHOR: Williams M R (Reprint); Arthur J S C; Balendran A; van der Kaay J; Poli V; Cohen P; Alessi D R
CORPORATE SOURCE: Univ Dundee, MRC, Prot Phosphorylat Unit, MSI-WTB Complex, Dow St, Wellcome Trust Bldg, Dundee DD1 5EH, Scotland (Reprint); Univ Dundee, MRC, Prot Phosphorylat Unit, Dundee DD1 5EH, Scotland; Univ Dundee, Dept Biochem, Dundee DD1 5EH, Scotland
COUNTRY OF AUTHOR: Scotland
SOURCE: CURRENT BIOLOGY, (20 APR 2000) Vol. 10, No. 8, pp. 439-448
ISSN: 0960-9822.
PUBLISHER: CELL PRESS, 1100 MASSACHUSETTES AVE,, CAMBRIDGE, MA 02138 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 52
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Protein kinase B (PKB), and the p70 and p90 ribosomal S6 kinases (p70 S6 kinase and p90 Rsk, respectively), are activated by phosphorylation of two residues, one in the 'T-loop' of the kinase domain and, the other, in the hydrophobic motif carboxy terminal to the kinase domain. The 3-**phosphoinositide**-dependent protein kinase 1 (**PDK1**) activates many AGC kinases in vitro by phosphorylating the T-loop residue, but whether **PDK1** also phosphorylates the hydrophobic motif and whether all other AGC kinases are substrates for **PDK1** is unknown.

Results: Mouse embryonic stem (ES) cells in which both copies of the **PDK1** gene were disrupted were viable. In **PDK2**(-/-) ES cells, PKB, p70 S6 kinase and p90 Rsk were not activated by stimuli that induced strong activation in **PDK1**(+/+) cells. Other AGC kinases - namely, protein kinase A (PKA), the mitogen- and stress-activated protein kinase 1 (MSK1) and the AMP-activated protein kinase (AMPK) - had normal activity or were activated normally in **PDK1**(-/-) cells. The insulin-like growth factor 1 (IGF1) induced PKB phosphorylation at its hydrophobic motif, but not at its T-loop residue, in **PDK1**(-/-) cells. IGF1 did not induce phosphorylation of p70 S6 kinase at its hydrophobic motif in **PDK1**(-/-) cells.

Conclusions: **PDK1** mediates activation of PKB, p70 S6 kinase and p90 Rsk in vivo, but is not rate-limiting for activation of PKA, MSK1 and AMPK. Another kinase phosphorylates PKB at its hydrophobic motif in **PDK1**(-/-) cells. **PDK1** phosphorylates the hydrophobic motif of p70 S6 kinase either directly or by activation of another kinase.

L12 ANSWER 21 OF 24 MEDLINE on STN
ACCESSION NUMBER: 1999350634 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10421571
TITLE: Kinase phosphorylation: Keeping it all in the family.
AUTHOR: Peterson R T; Schreiber S L

CORPORATE SOURCE: Howard Hughes Medical Institute, Departments of Chemistry and Chemical Biology and Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138, USA.
SOURCE: Current biology : CB, (1999 Jul 15) 9 (14) R521-4. Ref: 16
Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20020420
Entered Medline: 19991117

AB The identification of **PDK1** as a kinase that phosphorylates the AGC family of kinases led to a hunt for '**PDK2**', a hypothetical regulated kinase(s) that would be required for full activation of the AGC kinases. Recent findings suggest that the elusive **PDK2** may actually be a familiar kinase with an atypical associate.

L12 ANSWER 22 OF 24 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1999244939 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10226025
TITLE: **PDK1** acquires **PDK2** activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2.
AUTHOR: Balendran A; Casamayor A; Deak M; Paterson A; Gaffney P; Currie R; Downes C P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK.
SOURCE: Current biology : CB, (1999 Apr 22) 9 (8) 393-404.
Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20020420
Entered Medline: 19990601

AB BACKGROUND: Protein kinase B (PKB) is activated by phosphorylation of Thr308 and of Ser473. Thr308 is phosphorylated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) but the identity of the kinase that phosphorylates Ser473 (provisionally termed **PDK2**) is unknown. RESULTS: The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (PRK2), termed the **PDK1**-interacting fragment (PIF). PIF is situated carboxy-terminal to the kinase domain of PRK2, and contains a consensus motif for phosphorylation by **PDK2** similar to that found in PKBalpha, except that the residue equivalent to Ser473 is aspartic acid. Mutation of any of the conserved residues in the **PDK2** motif of PIF prevented interaction of PIF with **PDK1** . Remarkably, interaction of **PDK1** with PIF, or with a synthetic peptide encompassing the **PDK2** consensus sequence of PIF, converted **PDK1** from an enzyme that could phosphorylate only Thr308 of PKBalpha to one that phosphorylates both Thr308 and Ser473 of PKBalpha in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3). Furthermore, the interaction of PIF with **PDK1** converted the **PDK1** from a form that is not directly activated by PtdIns(3,4,5)P3 to a form that is activated threefold by PtdIns(3,4,5)P3. We have partially purified a kinase from brain extract that phosphorylates Ser473 of PKBalpha in a PtdIns(3,4,5)P3-dependent

manner and that is immunoprecipitated with **PDK1** antibodies. CONCLUSIONS: **PDK1** and **PDK2** might be the same enzyme, the substrate specificity and activity of **PDK1** being regulated through its interaction with another protein(s). PRK2 is a probable substrate for **PDK1**.

L12 ANSWER 23 OF 24 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1999208518 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10191262
TITLE: Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-**phosphoinositide** -dependent protein kinase-1 (**PDK1**) and **PDK2**.
AUTHOR: Kobayashi T; Cohen P
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, MSI/WTB Complex, Dow Street, Dundee DD1 5EH, Scotland, UK..
tkobayashi@bad.dundee.ac.uk
SOURCE: Biochemical journal, (1999 Apr 15) 339 (Pt 2) 319-28.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 20020420
Entered Medline: 19990623

AB The PtdIns(3,4,5)P3-dependent activation of protein kinase B (PKB) by 3-**phosphoinositide**-dependent protein kinases-1 and -2 (**PDK1** and **PDK2** respectively) is a key event in mediating the effects of signals that activate PtdIns 3-kinase. The catalytic domain of serum- and glucocorticoid-regulated protein kinase (SGK) is 54% identical with that of PKB and, although lacking the PtdIns(3,4, 5)P3-binding pleckstrin-homology domain, SGK retains the residues that are phosphorylated by **PDK1** and **PDK2**, which are Thr256 and Ser422 in SGK. Here we show that **PDK1** activates SGK in vitro by phosphorylating Thr256. We also show that, in response to insulin-like growth factor-1 (IGF-1) or hydrogen peroxide, transfected SGK is activated in 293 cells via a PtdIns 3-kinase-dependent pathway that involves the phosphorylation of Thr256 and Ser422. The activation of SGK by **PDK1** in vitro is unaffected by PtdIns(3,4,5)P3, abolished by the mutation of Ser422 to Ala, and greatly potentiated by mutation of Ser422 to Asp (although this mutation does not activate SGK itself). Consistent with these findings, the Ser422Asp mutant of SGK is activated by phosphorylation (probably at Thr256) in unstimulated 293 cells, and activation is unaffected by inhibitors of PtdIns 3-kinase. Our results are consistent with a model in which activation of SGK by IGF-1 or hydrogen peroxide is initiated by a PtdIns(3,4, 5)P3-dependent activation of **PDK2**, which phosphorylates Ser422. This is followed by the PtdIns(3,4,5)P3-independent phosphorylation at Thr256 that activates SGK, and is catalysed by **PDK1**. Like PKB, SGK preferentially phosphorylates serine and threonine residues that lie in Arg-Xaa-Arg-Xaa-Xaa-Ser/Thr motifs, and SGK and PKB inactivate glycogen synthase kinase-3 similarly in vitro and in co-transfection experiments. These findings raise the possibility that some physiological roles ascribed to PKB on the basis of the overexpression of constitutively active PKB mutants might be mediated by SGK.

L12 ANSWER 24 OF 24 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 1999061974 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9843996

TITLE: The akt kinase: molecular determinants of oncogenicity.
AUTHOR: Aoki M; Batista O; Bellacosa A; Tsichlis P; Vogt P K
CORPORATE SOURCE: Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, BCC239, La Jolla, CA 92037, USA.
CONTRACT NUMBER: CA42564 (NCI)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Dec 8) 95 (25) 14950-5. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990128
Last Updated on STN: 20020420
Entered Medline: 19990114

AB The serine-threonine kinase Akt is a downstream target of **phosphoinositide** 3-kinase (PI 3-kinase); it is activated by the **phosphoinositide** 3-phosphate-dependent kinases **PDK1** and **PDK2**. Certain mutated forms of Akt induce oncogenic transformation in chicken embryo fibroblast cultures and hemangiosarcomas in young chickens. This ability to transform cells depends on localization of Akt at the plasma membrane and on the kinase activity of Akt. A transdominant negative form of Akt interferes with oncogenic transformation induced by the p3k oncogene, which codes for an activated form of PI 3-kinase. Akt is therefore an essential mediator of p3k-induced oncogenicity.

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=> d his

     (FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005
L1          1799 S "PDK1"
L2         62622 S PHOSPHOINOSITIDE##
L3          1051 S L1 AND L2
L4          2934 S  "PIF" OR "PRK2"
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7
L9            35 S L3 AND PKBALPHA
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)
L11           67 S L3 AND "PDK2"
L12           24 DUP REM L11 (43 DUPLICATES REMOVED)

=> s ser473 or thr308
L13          884 SER473 OR THR308

=> s l12 and l13
L14            4 L12 AND L13

=> dup rem l14
PROCESSING COMPLETED FOR L14
L15            4 DUP REM L14 (0 DUPLICATES REMOVED)

=> d 1-4 ibib ab

L15  ANSWER 1 OF 4      MEDLINE on STN
ACCESSION NUMBER:   2004259252      MEDLINE
```

DOCUMENT NUMBER: PubMed ID: 15157674
TITLE: Regulation of protein kinase B/Akt activity and **Ser473** phosphorylation by protein kinase Calpha in endothelial cells.
AUTHOR: Partovian Chohreh; Simons Michael
CORPORATE SOURCE: Department of Medicine, Angiogenesis Research Center and Section of Cardiology, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756, USA.
CONTRACT NUMBER: HL62289 (NHLBI)
HL63609 (NHLBI)
SOURCE: Cellular signalling, (2004 Aug) 16 (8) 951-7.
Journal code: 8904683. ISSN: 0898-6568.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200505
ENTRY DATE: Entered STN: 20040526
Last Updated on STN: 20050520
Entered Medline: 20050519

AB Protein kinase Balpha (PKBalpha/Akt-1) is a key mediator of multiple signaling pathways involved in angiogenesis, cell proliferation and apoptosis among others. The unphosphorylated form of Akt-1 is virtually inactive and its full activation requires two phosphatidylinositol-3,4,5-triphosphate-dependent phosphorylation events, **Thr308** by 3-**phosphoinositide**-dependent kinase-1 (**PDK1**) and **Ser473** by an undefined kinase that has been termed **PDK2**. Recent studies have suggested that the **Ser473** kinase is a plasma membrane raft-associated kinase. In this study we show that protein kinase Calpha (PKCalpha) translocates to the membrane rafts in response to insulin growth factor-1 (IGF-1) stimulation. Overexpression of PKCalpha increases **Ser473** phosphorylation and Akt-1 activity, while inhibition of its activity or expression decreases IGF-1-dependent activation of Akt-1. Furthermore, in vitro, in the presence of phospholipids and calcium, PKCalpha directly phosphorylates Akt-1 at the **Ser473** site. We conclude, therefore, that PKCalpha regulates Akt-1 activity via **Ser473** phosphorylation and may function as **PDK2** in endothelial cells.

L15 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:258182 BIOSIS
DOCUMENT NUMBER: PREV200300258182
TITLE: Regulation of epithelial-mesenchymal transformation in palate development.
AUTHOR(S): Kang, Pei [Reprint Author]; Svoboda, Kathy K H
CORPORATE SOURCE: Baylor College of Dentistry, Texas AandM University Health Science Center, 3302 Gaston Ave, Dallas, TX, 75246, USA
pkang@tambcd.edu; ksvoboda@tambcd.edu
SOURCE: FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 718.5. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB.
ISSN: 0892-6638 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Jun 2003
Last Updated on STN: 4 Jun 2003

AB During development, palatal shelf medial edge epithelia (MEE) make contact, adhere and form desmosomes. The epithelia thin to one layer, lose cell-cell adhesion, degrade the basal lamina, become fusiform and

migrate into mesenchyme. This is termed epithelial-mesenchymal transformation (EMT). TGF[beta]3 and PI-3 kinase are essential for EMT. Alternatively, high doses of nicotine or its receptor antagonists block EMT and palatal fusion in vitro. TGF[beta]3 can signal through the Smad or the PI-3 kinase pathway. The nicotinic acetylcholine receptors (nAChRs) also signal through the PI-3 kinase pathway. Activiated PI-3 kinase stimulates **PDK1**/2 (integrin-linked kinase) that phosphorylates PKB/Akt, at serine 473. In this study the activity of PI-3 kinase was investigated by assaying the activation of Akt (**Ser473**). Palatal shelves from 13.5 day mouse embryos were cultured for 20 hrs in serum free media with or without 6 mM nicotine or LY294002 (1[mu]M, and 10[mu]M), a PI-3 kinase inhibitor. Akt(**ser473**) was immuno-localized on cryostat sections. Western blots of MEE isolated from whole cultured palates determined the amount of active Akt. Phosphorylated Akt was decreased in palates exposed to nicotine in western blots and immunolocalization studies. In conclusion, blocking PI-3 kinase or treating with nicotine decreased the activity of Akt. These observations provide evidence that the PI-3 kinase pathway may cross talk with nAChR signaling for EMT during palate fusion.

L15 ANSWER 3 OF 4 MEDLINE on STN
ACCESSION NUMBER: 2001517162 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11563975
TITLE: Tumour necrosis factor-alpha activation of protein kinase B in WEHI-164 cells is accompanied by increased phosphorylation of **Ser473**, but not **Thr308**.
AUTHOR: O'toole A; Moule S K; Lockyer P J; Halestrap A P
CORPORATE SOURCE: Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.
SOURCE: Biochemical journal, (2001 Oct 1) 359 (Pt 1) 119-27. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 20010924
Last Updated on STN: 20021211
Entered Medline: 20030807

AB Tumour necrosis factor-alpha (TNF-alpha) may activate both cell survival and cell death pathways. In the murine fibrosarcoma cell line WEHI-164, physiological concentrations (1 ng/ml) of TNF-alpha induced wortmannin-sensitive cell ruffling characteristic of the **phosphoinositide** 3-kinase (PI3-kinase) activation associated with cell survival. Wortmannin also enhanced cell death induced by TNF-alpha in the presence of actinomycin D, confirming that TNF-alpha activates a transcription-independent survival pathway requiring PI3-kinase activity. Both TNF-alpha and insulin-like growth factor 1 (IGF-1) caused a 6-10-fold wortmannin-sensitive increase in protein kinase B (PKB) activity within 5 min. For IGF-1, this was associated with an increase in phosphorylation of both Thr(308) and Ser(473), whereas for TNF-alpha only phosphorylation of Ser(473) was increased, even in the presence of okadaic acid to inhibit protein phosphatases 1 and 2A. TNF-alpha did not decrease the phosphorylation of Thr(308) induced by IGF-1, implying that TNF-alpha neither inhibits **phosphoinositide**-dependent kinase 1 (**PDK1**) nor activates an opposing phosphatase. In WEHI cells overexpressing a form of PKB, IGF-1 increased phosphorylation of Ser(473) on PKB, but not its kinase activity, whereas TNF-alpha failed to induce Ser(473) phosphorylation or kinase activation of either overexpressed T308A or wild-type PKB (where T308A is the mutant bearing the substitution Thr(308)-->A). IGF-1 caused translocation of green-fluorescent-protein-tagged ADP-ribosylation factor nucleotide-binding site opener (ARNO) to

the plasma membrane of WEHI cells, but this was not detected with TNF-alpha. We conclude that, at physiological concentrations, TNF-alpha activates endogenous PKB by stimulating **PDK2** (increase in Ser(473) phosphorylation) in a PI3-kinase-dependent (wortmannin-sensitive) manner, without causing detectable stimulation of **PDK1** (no increase in Thr(308) phosphorylation) or ARNO translocation. Possible explanations of these observations are discussed.

L15 ANSWER 4 OF 4 MEDLINE on STN
ACCESSION NUMBER: 1999244939 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10226025
TITLE: **PDK1** acquires **PDK2** activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2.
AUTHOR: Balendran A; Casamayor A; Deak M; Paterson A; Gaffney P; Currie R; Downes C P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK.
SOURCE: Current biology : CB, (1999 Apr 22) 9 (8) 393-404. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20020420
Entered Medline: 19990601

AB BACKGROUND: Protein kinase B (PKB) is activated by phosphorylation of **Thr308** and of **Ser473**. **Thr308** is phosphorylated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) but the identity of the kinase that phosphorylates **Ser473** (provisionally termed **PDK2**) is unknown. RESULTS: The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (PRK2), termed the **PDK1**-interacting fragment (PIF). PIF is situated carboxy-terminal to the kinase domain of PRK2, and contains a consensus motif for phosphorylation by **PDK2** similar to that found in PKBalpha, except that the residue equivalent to **Ser473** is aspartic acid. Mutation of any of the conserved residues in the **PDK2** motif of PIF prevented interaction of PIF with **PDK1**. Remarkably, interaction of **PDK1** with PIF, or with a synthetic peptide encompassing the **PDK2** consensus sequence of PIF, converted **PDK1** from an enzyme that could phosphorylate only **Thr308** of PKBalpha to one that phosphorylates both **Thr308** and **Ser473** of PKBalpha in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3). Furthermore, the interaction of PIF with **PDK1** converted the **PDK1** from a form that is not directly activated by PtdIns(3,4,5)P3 to a form that is activated threefold by PtdIns(3,4,5)P3. We have partially purified a kinase from brain extract that phosphorylates **Ser473** of PKBalpha in a PtdIns(3,4,5)P3-dependent manner and that is immunoprecipitated with **PDK1** antibodies. CONCLUSIONS: **PDK1** and **PDK2** might be the same enzyme, the substrate specificity and activity of **PDK1** being regulated through its interaction with another protein(s). PRK2 is a probable substrate for **PDK1**.

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E1            2     LESSI ALESSIO/AU
E2            1     LESSI CARLO/AU
E3            1 --> LESSI D/AU
E4            2     LESSI D R R P/AU
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E5            1     LESSI DULCE/AU
E6            9     LESSI E/AU
E7            4     LESSI EDSON/AU
E8            1     LESSI ELISABETTA/AU
E9           10     LESSI J/AU
E10           2     LESSI JACQUES/AU
E11          19     LESSI M/AU
E12           3     LESSI MONICA/AU

=> e alessi d/au
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E2            1     ALESSI CRISTIANE RODRIGUES/AU
E3          118 --> ALESSI D/AU
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E5            2     ALESSI D E/AU
E6            1     ALESSI D F/AU
E7            1     ALESSI D L/AU
E8           73     ALESSI D M/AU
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E11           3     ALESSI D S/AU
E12           4     ALESSI DANIEL/AU

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L16         118 "ALESSI D"/AU

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E5           15     BALENDRAN ANUDHARAN/AU
E6            8     BALENDRAN B/AU
E7            2     BALENDRAN C/AU
E8            3     BALENDRAN CLARE/AU
E9           22     BALENDRAN N/AU
E10           4     BALENDRAN NALINI/AU
E11          15     BALENDRAN P/AU
E12           2     BALENDRAN R/AU

=> s e3-e5
L17          45 ("BALENDRAN A"/AU OR "BALENDRAN ANU"/AU OR "BALENDRAN ANUDHARAN"
                /AU)

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E2            1     DEAK LR/AU
E3          183 --> DEAK M/AU
E4           13     DEAK M A/AU
E5            7     DEAK M M/AU
E6           13     DEAK M R/AU
E7            1     DEAK MAGDOLNA/AU
E8          136     DEAK MARIA/AU
E9            1     DEAK MARTIN/AU
E10           2     DEAK MARY ROSE/AU
E11           1     DEAK MARYANN C/AU
E12           1     DEAK MIHAIL/AU

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L18         353 ("DEAK M"/AU OR "DEAK M A"/AU OR "DEAK M M"/AU OR "DEAK M R"/AU
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=> e currie r/au
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E2            1      CURRIE PHILLIP J/AU
E3           99 --> CURRIE R/AU
E4          148      CURRIE R A/AU
E5            2      CURRIE R A */AU
E6           33      CURRIE R ALEXANDER/AU
E7           25      CURRIE R B/AU
E8            5      CURRIE R C/AU
E9           10      CURRIE R D/AU
E10           1      CURRIE R E/AU
E11          14      CURRIE R F/AU
E12          85      CURRIE R G/AU

=> s e3
L19          99 "CURRIE R"/AU

=> e downs p/au
E1            1      DOWNS O H/AU
E2            4      DOWNS O H J/AU
E3           79 --> DOWNS P/AU
E4           22      DOWNS P A/AU
E5           12      DOWNS P E/AU
E6            1      DOWNS P E JR/AU
E7            1      DOWNS P L/AU
E8            1      DOWNS P R/AU
E9           15      DOWNS P W/AU
E10           1      DOWNS PAMELA/AU
E11           2      DOWNS PATRICIA/AU
E12           1      DOWNS PAUL A/AU

=> e downes p/au
E1            3      DOWNES NINA/AU
E2            1      DOWNES NOEL/AU
E3           26 --> DOWNES P/AU
E4           10      DOWNES P C/AU
E5            4      DOWNES P J/AU
E6           22      DOWNES P K/AU
E7            6      DOWNES P M/AU
E8            2      DOWNES P S/AU
E9            3      DOWNES PATRICK/AU
E10           1      DOWNES PAUL/AU
E11           1      DOWNES PETE/AU
E12           8      DOWNES PETER/AU

=> s e3-e12
L20          83 ("DOWNES P"/AU OR "DOWNES P C"/AU OR "DOWNES P J"/AU OR "DOWNES
                P K"/AU OR "DOWNES P M"/AU OR "DOWNES P S"/AU OR "DOWNES PATRICK
                "/AU OR "DOWNES PAUL"/AU OR "DOWNES PETE"/AU OR "DOWNES PETER"/A
                U)

=> e casamayor a/au
E1            1      CASAMAYO J/AU
E2            1      CASAMAYOR/AU
E3          110 --> CASAMAYOR A/AU
E4            1      CASAMAYOR A J/AU
E5           60      CASAMAYOR ANTONIO/AU
E6            1      CASAMAYOR ANTONIO J/AU
E7            1      CASAMAYOR DAUDINOT R/AU
E8           12      CASAMAYOR DEL CACHO M/AU
E9            1      CASAMAYOR DUADINET R/AU
E10           1      CASAMAYOR E/AU
E11          67      CASAMAYOR E O/AU
E12          28      CASAMAYOR EMILIO O/AU
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=> s e3
L21         110 "CASAMAYOR A"/AU

=> d his

     (FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005
L1          1799 S "PDK1"
L2         62622 S PHOSPHOINOSITIDE##
L3          1051 S L1 AND L2
L4          2934 S  "PIF" OR "PRK2"
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7
L9            35 S L3 AND PKBALPHA
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)
L11           67 S L3 AND "PDK2"
L12           24 DUP REM L11 (43 DUPLICATES REMOVED)
L13          884 S SER473 OR THR308
L14            4 S L12 AND L13
L15            4 DUP REM L14 (0 DUPLICATES REMOVED)
                 E LESSI D/AU
                 E ALESSI D/AU
L16          118 S E3
                 E BALENDRAN A/AU
L17           45 S E3-E5
                 E DEAK M/AU
L18          353 S E3-E8
                 E CURRIE R/AU
L19           99 S E3
                 E DOWNS P/AU
                 E DOWNES P/AU
L20           83 S E3-E12
                 E CASAMAYOR A/AU
L21          110 S E3

=> s l16 or l17 or l18 or l19 or l20 or l21
L22         764 L16 OR L17 OR L18 OR L19 OR L20 OR L21

=> s l3 and l22
L23         126 L3 AND L22

=> s l13 and l23
L24          15 L13 AND L23

=> dup rem l24
PROCESSING COMPLETED FOR L24
L25           4 DUP REM L24 (11 DUPLICATES REMOVED)

=> d 1-4 ibib ab

L25   ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-01618  BIOTECHDS
TITLE:            Altering substrate specificity of phosphoinositide
                  -dependent protein-kinase-1, to phosphorylate Ser473
                  n addition to Thr308 by exposing to interacting
                  polypeptide;
                     drug screening
AUTHOR:           Alessi D; Balendran A; Deak M;
```

Currie R; Downes P; Casamayor A
PATENT ASSIGNEE: Univ.Dundee
LOCATION: Dundee, UK.
PATENT INFO: WO 2000056864 28 Sep 2000
APPLICATION INFO: WO 2000-GB1004 17 Mar 2000
PRIORITY INFO: GB 1999-6245 19 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-647155 [62]
AB Altering the substrate specificity of **phosphoinositide** -dependent protein-kinase-1 (**PDK1**) (I) by exposing it to an interacting protein (IP) is claimed. The IP has disclosed protein sequence. Also claimed are: a preparation (II) of (I) and an IP which comprises (S1), where (II) is free of proteins with which (I) is present in a cell in which it is naturally found, other than the IP or a substrate for (I); **PDK1** in which (I) has altered substrate specificity and is free of proteins with which (I) is present in a cell; a preparation of (I) and protein-kinase-C-related protein-kinase-2 in the absence of other proteins or cellular components; identifying a compound that modulates the activation and/or phosphorylation of protein-kinase-2 by (I), the activation and/or phosphorylation being measured in the presence of more than one concentration of the compound; drug screening methods; a protein-kinase from mammal brain; polynucleotides encoding protein-kinase proteins; a vector and transformed host cell and methods for preparing the recombinant proteins; therapy; and kits for screening. (103pp)

L25 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1999244939 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10226025
TITLE: **PDK1** acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2.
AUTHOR: **Balendran A**; **Casamayor A**; **Deak M**; Paterson A; Gaffney P; **Currie R**; Downes C P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK.
SOURCE: Current biology : CB, (1999 Apr 22) 9 (8) 393-404. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20020420
Entered Medline: 19990601
AB BACKGROUND: Protein kinase B (PKB) is activated by phosphorylation of **Thr308** and of **Ser473**. **Thr308** is phosphorylated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) but the identity of the kinase that phosphorylates **Ser473** (provisionally termed PDK2) is unknown. RESULTS: The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (PRK2), termed the **PDK1** -interacting fragment (PIF). PIF is situated carboxy-terminal to the kinase domain of PRK2, and contains a consensus motif for phosphorylation by PDK2 similar to that found in PKBalpha, except that the residue equivalent to **Ser473** is aspartic acid. Mutation of any of the conserved residues in the PDK2 motif of PIF prevented interaction of PIF with **PDK1**. Remarkably, interaction of **PDK1** with PIF, or with a synthetic peptide encompassing the PDK2 consensus sequence of PIF, converted **PDK1** from an enzyme that could phosphorylate only

Thr308 of PKBalpha to one that phosphorylates both **Thr308** and **Ser473** of PKBalpha in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3). Furthermore, the interaction of PIF with **PDK1** converted the **PDK1** from a form that is not directly activated by PtdIns(3,4,5)P3 to a form that is activated threefold by PtdIns(3,4,5)P3. We have partially purified a kinase from brain extract that phosphorylates **Ser473** of PKBalpha in a PtdIns(3,4,5)P3-dependent manner and that is immunoprecipitated with **PDK1** antibodies. CONCLUSIONS: **PDK1** and PDK2 might be the same enzyme, the substrate specificity and activity of **PDK1** being regulated through its interaction with another protein(s). PRK2 is a probable substrate for **PDK1**.

L25 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998180962 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9512493
TITLE: Activation of protein kinase B beta and gamma isoforms by insulin in vivo and by 3-**phosphoinositide**-dependent protein kinase-1 in vitro: comparison with protein kinase B alpha.
AUTHOR: Walker K S; **Deak M**; Paterson A; Hudson K; Cohen P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.. kswalker@BAD.dundee.ac.uk
SOURCE: Biochemical journal, (1998 Apr 1) 331 (Pt 1) 299-308. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 20020420
Entered Medline: 19980513

AB The regulatory and catalytic properties of the three mammalian isoforms of protein kinase B (PKB) have been compared. All three isoforms (PKBalpha, PKBbeta and PKBgamma) were phosphorylated at similar rates and activated to similar extents by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**). Phosphorylation and activation of each enzyme required the presence of PtdIns(3,4,5)P3 or PtdIns(3,4)P2, as well as **PDK1**. The activation of PKBbeta and PKBgamma by **PDK1** was accompanied by the phosphorylation of the residues equivalent to **Thr308** in PKBalpha, namely Thr309 (PKBbeta) and Thr305 (PKBgamma). PKBgamma which had been activated by **PDK1** possessed a substrate specificity identical with that of PKBalpha and PKBbeta towards a range of peptides. The activation of PKBgamma and its phosphorylation at Thr305 was triggered by insulin-like growth factor-1 in 293 cells. Stimulation of rat adipocytes or rat hepatocytes with insulin induced the activation of PKBalpha and PKBbeta with similar kinetics. After stimulation of adipocytes, the activity of PKBbeta was twice that of PKBalpha, but in hepatocytes PKBalpha activity was four-fold higher than PKBbeta. Insulin induced the activation of PKBalpha in rat skeletal muscle in vivo, with little activation of PKBbeta. Insulin did not induce PKBgamma activity in adipocytes, hepatocytes or skeletal muscle, but PKBgamma was the major isoform activated by insulin in rat L6 myotubes (a skeletal-muscle cell line).

L25 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1998035195 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9368760
TITLE: 3-**Phosphoinositide**-dependent protein kinase-1 (**PDK1**): structural and functional homology with the

Drosophila DSTPK61 kinase.

AUTHOR: Alessi D R; **Deak M**; **Casamayor A**; Caudwell F B; Morrice N; Norman D G; Gaffney P; Reese C B; MacDougall C N; Harbison D; Ashworth A; Bownes M

CORPORATE SOURCE: Department of Biochemistry, University of Dundee, UK.. dralessi@bad.dundee.ac.uk

SOURCE: Current biology : CB, (1997 Oct 1) 7 (10) 776-89. Journal code: 9107782. ISSN: 0960-9822.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF017995

ENTRY MONTH: 199802

ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 20020420
Entered Medline: 19980210

AB BACKGROUND: The activation of protein kinase B (PKB, also known as c-Akt) is stimulated by insulin or growth factors and results from its phosphorylation at **Thr308** and **Ser473**. We recently identified a protein kinase, termed **PDK1**, that phosphorylates PKB at **Thr308** only in the presence of lipid vesicles containing phosphatidylinositol 3,4,5-trisphosphate (Ptdlns(3,4,5)P3) or phosphatidylinositol 3,4-bisphosphate (Ptdlns(3,4)P2). RESULTS: We have cloned and sequenced human **PDK1**. The 556-residue monomeric enzyme comprises a catalytic domain that is most similar to the PKA, PKB and PKC subfamily of protein kinases and a carboxy-terminal pleckstrin homology (PH) domain. The **PDK1** gene is located on human chromosome 16p13.3 and is expressed ubiquitously in human tissues. Human **PDK1** is homologous to the Drosophila protein kinase DSTPK61, which has been implicated in the regulation of sex differentiation, oogenesis and spermatogenesis. Expressed **PDK1** and DSTPK61 phosphorylated **Thr308** of PKB alpha only in the presence of Ptdlns(3,4,5)P3 or Ptdlns(3,4)P2. Overexpression of **PDK1** in 293 cells activated PKB alpha and potentiated the IGF1-induced phosphorylation of PKB alpha at **Thr308**. Experiments in which the PH domains of either **PDK1** or PKB alpha were deleted indicated that the binding of Ptdlns(3,4,5)P3 or Ptdlns(3,4)P2 to PKB alpha is required for phosphorylation and activation by **PDK1**. IGF1 stimulation of 293 cells did not affect the activity or phosphorylation of **PDK1**. CONCLUSIONS: **PDK1** is likely to mediate the activation of PKB by insulin or growth factors. DSTPK61 is a Drosophila homologue of **PDK1**. The effect of Ptdlns(3,4,5)P3/Ptdlns(3,4)P2 in the activation of PKB alpha is at least partly substrate directed.

=> d his

(FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005

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L1          1799 S "PDK1"
L2         62622 S PHOSPHOINOSITIDE##
L3          1051 S L1 AND L2
L4          2934 S  "PIF" OR "PRK2"
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7
L9            35 S L3 AND PKBALPHA
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)
L11           67 S L3 AND "PDK2"
```

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L12          24 DUP REM L11 (43 DUPLICATES REMOVED)
L13         884 S SER473 OR THR308
L14           4 S L12 AND L13
L15           4 DUP REM L14 (0 DUPLICATES REMOVED)
                E LESSI D/AU
                E ALESSI D/AU
L16         118 S E3
                E BALENDRAN A/AU
L17          45 S E3-E5
                E DEAK M/AU
L18         353 S E3-E8
                E CURRIE R/AU
L19          99 S E3
                E DOWNS P/AU
                E DOWNES P/AU
L20          83 S E3-E12
                E CASAMAYOR A/AU
L21         110 S E3
L22         764 S L16 OR L17 OR L18 OR L19 OR L20 OR L21
L23         126 S L3 AND L22
L24          15 S L13 AND L23
L25           4 DUP REM L24 (11 DUPLICATES REMOVED)

=> s s 19 and 122
MISSING OPERATOR S L9
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=>  s 19 and 122
L26          16 L9 AND L22

=> dup rem 126
PROCESSING COMPLETED FOR L26
L27           9 DUP REM L26 (7 DUPLICATES REMOVED)

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L27  ANSWER 1 OF 9     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2003493613     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 12964941
TITLE:               Binding of phosphatidylinositol 3,4,5-trisphosphate to the
                     pleckstrin homology domain of protein kinase B induces a
                     conformational change.
AUTHOR:              Milburn Christine C; Deak Maria; Kelly Sharon M;
                     Price Nick C; Alessi Dario R; Van Aalten Daan M F
CORPORATE SOURCE:    Division of Biological Chemistry and Molecular
                     Microbiology, School of Life Sciences, University of
                     Dundee, Dundee DD1 5EH, UK.
SOURCE:              Biochemical journal, (2003 Nov 1) 375 (Pt 3) 531-8.
                     Journal code: 2984726R. ISSN: 1470-8728.
PUB. COUNTRY:        England: United Kingdom
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
OTHER SOURCE:        PDB-1UNP; PDB-1UNQ; PDB-1UNR
ENTRY MONTH:         200404
ENTRY DATE:          Entered STN: 20031023
                     Last Updated on STN: 20040427
                     Entered Medline: 20040426
AB   Protein kinase B (PKB/Akt) is a key regulator of cell growth,
     proliferation and metabolism.  It possesses an N-terminal pleckstrin
     homology (PH) domain that interacts with equal affinity with the second
     messengers PtdIns(3,4,5)P3 and PtdIns(3,4)P2, generated through insulin
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and growth factor-mediated activation of **phosphoinositide** 3-kinase (PI3K). The binding of PKB to PtdIns(3,4,5)P3/PtdIns(3,4)P2 recruits PKB from the cytosol to the plasma membrane and is also thought to induce a conformational change that converts PKB into a substrate that can be activated by the **phosphoinositide**-dependent kinase 1 (**PDK1**). In this study we describe two high-resolution crystal structures of the PH domain of **PKBalpha** in a noncomplexed form and compare this to a new atomic resolution (0.98 A, where 1 A=0.1 nm) structure of the PH domain of **PKBalpha** complexed to Ins(1,3,4,5)P4, the head group of PtdIns(3,4,5)P3. Remarkably, in contrast to all other PH domains crystallized so far, our data suggest that binding of Ins(1,3,4,5)P4 to the PH domain of PKB, induces a large conformational change. This is characterized by marked changes in certain residues making up the **phosphoinositide**-binding site, formation of a short a-helix in variable loop 2, and a movement of variable loop 3 away from the lipid-binding site. Solution studies with CD also provided evidence of conformational changes taking place upon binding of Ins(1,3,4,5)P4 to the PH domain of PKB. Our data provides the first structural insight into the mechanism by which the interaction of PKB with PtdIns(3,4,5)P3/PtdIns(3,4)P2 induces conformational changes that could enable PKB to be activated by **PDK1**.

L27 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002658121 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12374740
TITLE: A phosphoserine/threonine-binding pocket in AGC kinases and **PDK1** mediates activation by hydrophobic motif phosphorylation.
AUTHOR: Frodin Morten; Antal Torben L; Dummler Bettina A; Jensen Claus J; **Deak Maria**; Gammeltoft Steen; Biondi Ricardo M
CORPORATE SOURCE: Department of Clinical Biochemistry, Glostrup Hospital, DK-2600 Glostrup, Denmark.. mf@dcb-glostrup.dk
SOURCE: EMBO journal, (2002 Oct 15) 21 (20) 5396-407. Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20021107
Last Updated on STN: 20021214
Entered Medline: 20021126

AB The growth factor-activated AGC protein kinases RSK, S6K, PKB, MSK and SGK are activated by serine/threonine phosphorylation in the activation loop and in the hydrophobic motif, C-terminal to the kinase domain. In some of these kinases, phosphorylation of the hydrophobic motif creates a specific docking site that recruits and activates **PDK1**, which then phosphorylates the activation loop. Here, we discover a pocket in the kinase domain of **PDK1** that recognizes the phosphoserine/phosphothreonine in the hydrophobic motif by identifying two oppositely positioned arginine and lysine residues that bind the phosphate. Moreover, we demonstrate that RSK2, S6K1, **PKBalpha**, MSK1 and SGK1 contain a similar phosphate-binding pocket, which they use for intramolecular interaction with their own phosphorylated hydrophobic motif. Molecular modelling and experimental data provide evidence for a common activation mechanism in which the phosphorylated hydrophobic motif and activation loop act on the alphaC-helix of the kinase structure to induce synergistic stimulation of catalytic activity. Sequence conservation suggests that this mechanism is a key feature in activation of >40 human AGC kinases.

L27 ANSWER 3 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:468641 BIOSIS
DOCUMENT NUMBER: PREV200200468641
TITLE: High-resolution structure of the pleckstrin homology domain of protein kinase B/Akt bound to phosphatidylinositol (3,4,5)-trisphosphate.
AUTHOR(S): Thomas, Christine C.; **Deak, Maria**; Alessi, Dario R.; van Aalten, Daan M. F. [Reprint author]
CORPORATE SOURCE: Division of Biological Chemistry and Molecular Microbiology, University of Dundee, Dundee, DD1 5EH, UK dava@davapc1.bioch.dundee.ac.uk
SOURCE: Current Biology, (July 23, 2002) Vol. 12, No. 14, pp. 1256-1262. print.
CODEN: CUBLE2. ISSN: 0960-9822.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Sep 2002
Last Updated on STN: 4 Sep 2002

AB The products of PI 3-kinase activation, PtdIns(3,4,5)P3 and its immediate breakdown product PtdIns(3,4)P2, trigger physiological processes, by interacting with proteins possessing pleckstrin homology (PH) domains. One of the best characterized PtdIns(3,4,5)P3/PtdIns(3,4)P2 effector proteins is protein kinase B (PKB), also known as Akt. PKB possesses a PH domain located at its N terminus, and this domain binds specifically to PtdIns(3,4,5)P3 and PtdIns(3,4)P2 with similar affinity. Following activation of PI 3-kinase, PKB is recruited to the plasma membrane by virtue of its interaction with PtdIns(3,4,5)P3/PtdIns(3,4)P2. PKB is then activated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**), which like PKB, possesses a PtdIns(3,4,5)P3/PtdIns(3,4)P2 binding PH domain. Here, we describe the high-resolution crystal structure of the isolated PH domain of **PKBalpha** in complex with the head group of PtdIns(3,4,5)P3. The head group has a significantly different orientation and location compared to other Ins(1,3,4,5)P4 binding PH domains. Mutagenesis of the basic residues that form ionic interactions with the D3 and D4 phosphate groups reduces or abolishes the ability of PKB to interact with PtdIns(3,4,5)P3 and PtdIns(3,4)P2. The D5 phosphate faces the solvent and forms no significant interactions with any residue on the PH domain, and this explains why PKB interacts with similar affinity with both PtdIns(3,4,5)P3 and PtdIns(3,4)P2.

L27 ANSWER 4 OF 9 MEDLINE on STN
ACCESSION NUMBER: 2001454762 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11500365
TITLE: The PIF-binding pocket in **PDK1** is essential for activation of S6K and SGK, but not PKB.
AUTHOR: Biondi R M; Kieloch A; Currie R A; **Deak M**; Alessi D R
CORPORATE SOURCE: Division of Signal Transduction Therapy, MRC Protein Phosphorylation Unit, School of Life Sciences, MSI/WTB complex, University of Dundee, Dow Street, Dundee DD1 5EH, UK.. r.m.biondi@dundee.ac.uk
SOURCE: EMBO journal, (2001 Aug 15) 20 (16) 4380-90.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010814
Last Updated on STN: 20020420
Entered Medline: 20011025

AB PKB/Akt, S6K1 and SGK are related protein kinases activated in a PI 3-kinase-dependent manner in response to insulin/growth factors signalling. Activation entails phosphorylation of these kinases at two

residues, the T-loop and the hydrophobic motif. **PDK1** activates S6K, SGK and PKB isoforms by phosphorylating these kinases at their T-loop. We demonstrate that a pocket in the kinase domain of **PDK1**, termed the 'PIF-binding pocket', plays a key role in mediating the interaction and phosphorylation of S6K1 and SGK1 at their T-loop motif by **PDK1**. Our data indicate that prior phosphorylation of S6K1 and SGK1 at their hydrophobic motif promotes their interaction with the PIF-binding pocket of **PDK1** and their T-loop phosphorylation. Thus, the hydrophobic motif phosphorylation of S6K and SGK converts them into substrates that can be activated by **PDK1**. In contrast, the PIF-binding pocket of **PDK1** is not required for the phosphorylation of **PKBalpha** by **PDK1**. The PIF-binding pocket represents a substrate recognition site on a protein kinase that is only required for the phosphorylation of a subset of its physiological substrates.

L27 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000069735 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10601311
TITLE: Evidence that 3-**phosphoinositide**-dependent protein kinase-1 mediates phosphorylation of p70 S6 kinase in vivo at Thr-412 as well as Thr-252.
AUTHOR: **Balendran A**; **Currie R**; Armstrong C G; Avruch J; Alessi D R
CORPORATE SOURCE: Medical Research Council Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, Scotland.
SOURCE: Journal of biological chemistry, (1999 Dec 24) 274 (52) 37400-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20020420
Entered Medline: 20000113

AB Protein kinase B and p70 S6 kinase are members of the cyclic AMP-dependent/cyclic GMP-dependent/protein kinase C subfamily of protein kinases and are activated by a phosphatidylinositol 3-kinase-dependent pathway when cells are stimulated with insulin or growth factors. Both of these kinases are activated in cells by phosphorylation of a conserved residue in the kinase domain (Thr-308 of protein kinase B (PKB) and Thr-252 of p70 S6 kinase) and another conserved residue located C-terminal to the kinase domain (Ser-473 of PKB and Thr-412 of p70 S6 kinase). Thr-308 of **PKBalpha** and Thr-252 of p70 S6 kinase are phosphorylated by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) in vitro. Recent work has shown that **PDK1** interacts with a region of protein kinase C-related kinase-2, termed the **PDK1** interacting fragment (PIF). Interaction with PIF converts **PDK1** from a form that phosphorylates PKB at Thr-308 alone to a species capable of phosphorylating Ser-473 as well as Thr-308. This suggests that **PDK1** may be the enzyme that phosphorylates both residues in vivo. Here we demonstrate that **PDK1** is capable of phosphorylating p70 S6 kinase at Thr-412 in vitro. We study the effect of PIF on the ability of **PDK1** to phosphorylate p70 S6 kinase. Surprisingly, we find that **PDK1** bound to PIF is no longer able to interact with or phosphorylate p70 S6 kinase in vitro at either Thr-252 or Thr-412. The expression of PIF in cells prevents insulin-like growth factor 1 from inducing the activation of the p70 S6 kinase and its phosphorylation at Thr-412. Overexpression of **PDK1** in cells induces the phosphorylation of p70 S6 kinase at Thr-412 in unstimulated

cells, and a catalytically inactive mutant of **PDK1** prevents the phosphorylation of p70 S6K at Thr-412 in insulin-like growth factor 1-stimulated cells. These observations indicate that **PDK1** regulates the activation of p70 S6 kinase and provides evidence that **PDK1** mediates the phosphorylation of p70 S6 kinase at Thr-412.

L27 ANSWER 6 OF 9 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999112925 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9895304
TITLE: Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-**phosphoinositide**-dependent protein kinase-1.
AUTHOR: Currie R A; Walker K S; Gray A; **Deak M**; **Casamayor A**; Downes C P; Cohen P; Alessi D R; Lucocq J
CORPORATE SOURCE: Department of Biochemistry, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, U.K.. racurrie@bad.dundee.ac.uk
SOURCE: Biochemical journal, (1999 Feb 1) 337 (Pt 3) 575-83. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990413
Last Updated on STN: 20020420
Entered Medline: 19990330

AB 3-**Phosphoinositide**-dependent protein kinase-1 (**PDK1**) interacts stereoselectively with the d-enantiomer of PtdIns(3,4,5)P3 (KD 1.6 nM) and PtdIns(3,4)P2 (KD 5.2 nM), but binds with lower affinity to PtdIns3P or PtdIns(4,5)P2. The binding of PtdIns(3,4,5)P3 to **PDK1** was greatly decreased by making specific mutations in the pleckstrin homology (PH) domain of **PDK1** or by deleting it. The same mutations also greatly decreased the rate at which **PDK1** activated protein kinase Balpha (**PKBalpha**) in vitro in the presence of lipid vesicles containing PtdIns(3,4,5)P3, but did not affect the rate at which **PDK1** activated a **PKBalpha** mutant lacking the PH domain in the absence of PtdIns(3,4,5)P3. When overexpressed in 293 or PAE cells, **PDK1** was located at the plasma membrane and in the cytosol, but was excluded from the nucleus. Mutations that disrupted the interaction of PtdIns(3,4,5)P3 or PtdIns(4,5)P2 with **PDK1** abolished the association of **PDK1** with the plasma membrane. Growth-factor stimulation promoted the translocation of transfected **PKBalpha** to the plasma membrane, but had no effect on the subcellular distribution of **PDK1** as judged by immunoelectron microscopy of fixed cells. This conclusion was also supported by confocal microscopy of green fluorescent protein-**PDK1** in live cells. These results, together with previous observations, indicate that PtdIns(3,4,5)P3 plays several roles in the **PDK1**-induced activation of **PKBalpha**. First, it binds to the PH domain of PKB, altering its conformation so that it can be activated by **PDK1**. Secondly, interaction with PtdIns(3,4,5)P3 recruits PKB to the plasma membrane with which **PDK1** is localized constitutively by virtue of its much stronger interaction with PtdIns(3,4,5)P3 or PtdIns(4,5)P2. Thirdly, the interaction of **PDK1** with PtdIns(3,4,5)P3 facilitates the rate at which it can activate PKB.

L27 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999244939 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10226025
TITLE: **PDK1** acquires PDK2 activity in the presence of a

synthetic peptide derived from the carboxyl terminus of PRK2.

AUTHOR: **Balendran A**; **Casamayor A**; **Deak M**; Paterson A; Gaffney P; **Currie R**; Downes C P; Alessi D R

CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK.

SOURCE: Current biology : CB, (1999 Apr 22) 9 (8) 393-404. Journal code: 9107782. ISSN: 0960-9822.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199906

ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20020420
Entered Medline: 19990601

AB BACKGROUND: Protein kinase B (PKB) is activated by phosphorylation of Thr308 and of Ser473. Thr308 is phosphorylated by the 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) but the identity of the kinase that phosphorylates Ser473 (provisionally termed PDK2) is unknown. RESULTS: The kinase domain of **PDK1** interacts with a region of protein kinase C-related kinase-2 (PRK2), termed the **PDK1**-interacting fragment (PIF). PIF is situated carboxy-terminal to the kinase domain of PRK2, and contains a consensus motif for phosphorylation by PDK2 similar to that found in **PKBalpha**, except that the residue equivalent to Ser473 is aspartic acid. Mutation of any of the conserved residues in the PDK2 motif of PIF prevented interaction of PIF with **PDK1**. Remarkably, interaction of **PDK1** with PIF, or with a synthetic peptide encompassing the PDK2 consensus sequence of PIF, converted **PDK1** from an enzyme that could phosphorylate only Thr308 of **PKBalpha** to one that phosphorylates both Thr308 and Ser473 of **PKBalpha** in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3). Furthermore, the interaction of PIF with **PDK1** converted the **PDK1** from a form that is not directly activated by PtdIns(3,4,5)P3 to a form that is activated threefold by PtdIns(3,4,5)P3. We have partially purified a kinase from brain extract that phosphorylates Ser473 of **PKBalpha** in a PtdIns(3,4,5)P3-dependent manner and that is immunoprecipitated with **PDK1** antibodies. CONCLUSIONS: **PDK1** and PDK2 might be the same enzyme, the substrate specificity and activity of **PDK1** being regulated through its interaction with another protein(s). PRK2 is a probable substrate for **PDK1**.

L27 ANSWER 8 OF 9 MEDLINE on STN

ACCESSION NUMBER: 1999175477 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10074427

TITLE: Functional counterparts of mammalian protein kinases **PDK1** and SGK in budding yeast.

AUTHOR: **Casamayor A**; Torrance P D; Kobayashi T; Thorner J; Alessi D R

CORPORATE SOURCE: MRC Protein Phosphorylation Unit Department of Biochemistry University of Dundee Dundee DD1 5EH Scotland UK.

CONTRACT NUMBER: GM21841 (NIGMS)

SOURCE: Current biology : CB, (1999 Feb 25) 9 (4) 186-97. Journal code: 9107782. ISSN: 0960-9822.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199904

ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 20020420

Entered Medline: 19990422

AB BACKGROUND: In animal cells, recruitment of phosphatidylinositol 3-kinase by growth factor receptors generates 3-**phosphoinositides**, which stimulate 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**). Activated **PDK1** then phosphorylates and activates downstream protein kinases, including protein kinase B (PKB)/c-Akt, p70 S6 kinase, PKC isoforms, and serum- and glucocorticoid-inducible kinase (SGK), thereby eliciting physiological responses. RESULTS: We found that two previously uncharacterised genes of Saccharomyces cerevisiae, which we term PKH1 and PKH2, encode protein kinases with catalytic domains closely resembling those of human and Drosophila **PDK1**. Both Pkh1 and Pkh2 were essential for cell viability. Expression of human **PDK1** in otherwise inviable pkh1Delta pkh2Delta cells permitted growth. In addition, the yeast YPK1 and YKR2 genes were found to encode protein kinases each with a catalytic domain closely resembling that of SGK; both Ypk1 and Ykr2 were also essential for viability. Otherwise inviable ypk1Delta ykr2Delta cells were fully rescued by expression of rat SGK, but not mouse PKB or rat p70 S6 kinase. Purified Pkh1 activated mammalian SGK and **PKBalpha** in vitro by phosphorylating the same residue as **PDK1**. Pkh1 activated purified Ypk1 by phosphorylating the equivalent residue (Thr504) and was required for maximal Ypk1 phosphorylation in vivo. Unlike PKB, activation of Ypk1 and SGK by Pkh1 did not require phosphatidylinositol 3,4,5-trisphosphate, consistent with the absence of pleckstrin homology domains in these proteins. The phosphorylation consensus sequence for Ypk1 was similar to that for **PKBalpha** and SGK. CONCLUSIONS: Pkh1 and Pkh2 function similarly to **PDK1**, and Ypk1 and Ykr2 to SGK. As in animal cells, these two groups of yeast kinases constitute two tiers of a signalling cascade required for yeast cell growth.

L27 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 1998180962 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9512493
TITLE: Activation of protein kinase B beta and gamma isoforms by insulin in vivo and by 3-**phosphoinositide** -dependent protein kinase-1 in vitro: comparison with protein kinase B alpha.
AUTHOR: Walker K S; **Deak M**; Paterson A; Hudson K; Cohen P; Alessi D R
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.. kswalker@BAD.dundee.ac.uk
SOURCE: Biochemical journal, (1998 Apr 1) 331 (Pt 1) 299-308. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 20020420
Entered Medline: 19980513

AB The regulatory and catalytic properties of the three mammalian isoforms of protein kinase B (PKB) have been compared. All three isoforms (**PKBalpha**, PKBbeta and PKBgamma) were phosphorylated at similar rates and activated to similar extents by 3-**phosphoinositide** -dependent protein kinase-1 (**PDK1**). Phosphorylation and activation of each enzyme required the presence of PtdIns(3,4,5)P3 or PtdIns(3,4)P2, as well as **PDK1**. The activation of PKBbeta and PKBgamma by **PDK1** was accompanied by the phosphorylation of the residues equivalent to Thr308 in **PKBalpha**, namely Thr309 (PKBbeta) and Thr305 (PKBgamma). PKBgamma which had been activated by **PDK1** possessed a substrate specificity identical with that of

PKBalpha and PKBbeta towards a range of peptides. The activation of PKBgamma and its phosphorylation at Thr305 was triggered by insulin-like growth factor-1 in 293 cells. Stimulation of rat adipocytes or rat hepatocytes with insulin induced the activation of **PKBalpha** and PKBbeta with similar kinetics. After stimulation of adipocytes, the activity of PKBbeta was twice that of **PKBalpha**, but in hepatocytes **PKBalpha** activity was four-fold higher than PKBbeta. Insulin induced the activation of **PKBalpha** in rat skeletal muscle in vivo, with little activation of PKBbeta. Insulin did not induce PKBgamma activity in adipocytes, hepatocytes or skeletal muscle, but PKBgamma was the major isoform activated by insulin in rat L6 myotubes (a skeletal-muscle cell line).

=> d his

(FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005

```
L1          1799 S "PDK1"
L2         62622 S PHOSPHOINOSITIDE##
L3          1051 S L1 AND L2
L4          2934 S  "PIF" OR "PRK2"
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7
L9            35 S L3 AND PKBALPHA
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)
L11           67 S L3 AND "PDK2"
L12           24 DUP REM L11 (43 DUPLICATES REMOVED)
L13          884 S SER473 OR THR308
L14            4 S L12 AND L13
L15            4 DUP REM L14 (0 DUPLICATES REMOVED)
                 E LESSI D/AU
                 E ALESSI D/AU
L16          118 S E3
                 E BALENDRAN A/AU
L17           45 S E3-E5
                 E DEAK M/AU
L18          353 S E3-E8
                 E CURRIE R/AU
L19           99 S E3
                 E DOWNS P/AU
                 E DOWNES P/AU
L20           83 S E3-E12
                 E CASAMAYOR A/AU
L21          110 S E3
L22          764 S L16 OR L17 OR L18 OR L19 OR L20 OR L21
L23          126 S L3 AND L22
L24           15 S L13 AND L23
L25            4 DUP REM L24 (11 DUPLICATES REMOVED)
L26           16 S L9 AND L22
L27            9 DUP REM L26 (7 DUPLICATES REMOVED)
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=> d his

     (FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005
L1          1799 S "PDK1"
L2         62622 S PHOSPHOINOSITIDE##
L3          1051 S L1 AND L2
L4          2934 S  "PIF" OR "PRK2"
L5            78 S L3 AND L4
L6            24 DUP REM L5 (54 DUPLICATES REMOVED)
L7           528 S "SERINE 473"
L8             0 S L6 AND L7
L9            35 S L3 AND PKBALPHA
L10           19 DUP REM L9 (16 DUPLICATES REMOVED)
L11           67 S L3 AND "PDK2"
L12           24 DUP REM L11 (43 DUPLICATES REMOVED)
L13          884 S SER473 OR THR308
L14            4 S L12 AND L13
L15            4 DUP REM L14 (0 DUPLICATES REMOVED)
                 E LESSI D/AU
                 E ALESSI D/AU
L16          118 S E3
                 E BALENDRAN A/AU
L17           45 S E3-E5
                 E DEAK M/AU
L18          353 S E3-E8
                 E CURRIE R/AU
L19           99 S E3
                 E DOWNS P/AU
                 E DOWNES P/AU
L20           83 S E3-E12
                 E CASAMAYOR A/AU
L21          110 S E3
L22          764 S L16 OR L17 OR L18 OR L19 OR L20 OR L21
L23          126 S L3 AND L22
L24           15 S L13 AND L23
L25            4 DUP REM L24 (11 DUPLICATES REMOVED)
L26           16 S L9 AND L22
L27            9 DUP REM L26 (7 DUPLICATES REMOVED)
L28           37 S L3 AND AGONIST?
L29           13 DUP REM L28 (24 DUPLICATES REMOVED)

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=> d 1-13 ibib ab

L29 ANSWER 1 OF 13 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005231519 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15867396
TITLE: Peroxisome proliferator-activated receptor delta and gamma **agonists** differentially alter tumor differentiation and progression during mammary carcinogenesis.
COMMENT: Erratum in: Cancer Res. 2005 Jul 1;65(13):5989
AUTHOR: Yin Yuzhi; Russell Robert G; Dettin Luis E; Bai Renkui; Wei Zhi-Liang; Kozikowski Alan P; Kopleovich Levy; Glazer Robert I
CORPORATE SOURCE: Department of Oncology, Georgetown University, Washington, District of Columbia 20057, USA.
CONTRACT NUMBER: N01-CN-25101 (NCI)
SOURCE: Cancer research, (2005 May 1) 65 (9) 3950-7. Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 20050504
Last Updated on STN: 20050616
Entered Medline: 20050615

AB Peroxisome proliferator-activated receptor (PPAR) represents a ligand-dependent nuclear receptor family that regulates multiple metabolic processes associated with fatty acid beta-oxidation, glucose utilization, and cholesterol transport. These and other receptor-mediated actions pertain to their role in hypolipidemic and antidiabetic therapies and as potential targets for cancer chemopreventive agents. The present study evaluated the chemopreventive activity of two highly potent and selective PPARgamma and PPARdelta **agonists** in a progestin- and carcinogen-induced mouse mammary tumorigenesis model. Animals treated with the PPARgamma **agonist** GW7845 exhibited a moderate delay in tumor formation. In contrast, animals treated with the PPARdelta **agonist** GW501516 showed accelerated tumor formation. Significantly, tumors from GW7845-treated mice were predominantly ductal adenocarcinomas, whereas tumors from GW501516-treated animals were adenosquamous and squamous cell carcinomas. Gene expression analysis of tumors arising from GW7845- and GW501516-treated mice identified expression profiles that were distinct from each other and from untreated control tumors of the same histopathology. Only tumors from mice treated with the PPARgamma **agonist** expressed estrogen receptor-alpha in luminal transit cells, suggesting increased ductal progenitor cell expansion. Tumors from mice treated with the PPARdelta **agonist** exhibited increased PPARdelta levels and activated 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**), which co-associated, suggesting a link between the known oncogenic activity of **PDK1** in mammary epithelium and PPARdelta activation. These results indicate that PPARdelta and PPARgamma **agonists** produce diverse, yet profound effects on mammary tumorigenesis that give rise to distinctive histopathologic patterns of tumor differentiation and tumor development.

L29 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:792557 HCAPLUS
DOCUMENT NUMBER: 139:290290
TITLE: Akt-mediated Cardiomyocyte Survival Pathways Are Compromised by Gαq-induced **Phosphoinositide** 4,5-Bisphosphate Depletion
AUTHOR(S): Howes, Amy L.; Arthur, Jane F.; Zhang, Tong; Miyamoto,

Shigeki; Adams, John W.; Dorn, Gerald W., II; Woodcock, Elizabeth A.; Brown, Joan Heller

CORPORATE SOURCE: Department of Pharmacology, University of California, San Diego, La Jolla, CA, 92093, USA

SOURCE: Journal of Biological Chemistry (2003), 278(41), 40343-40351
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Expression of the wild type α subunit of Gq (GqWT) in cardiomyocytes induces hypertrophy, whereas a constitutively active Gαq subunit (GqQ209L) induces apoptosis. Akt phosphorylation increases with GqWT expression but is markedly attenuated in cardiomyocytes expressing GqQ209L or in those expressing GqWT and treated with **agonist**. A membrane-targeted Akt rescues GqQ209L-expressing cardiomyocytes from apoptotic cell death. In contrast, leukemia inhibitory factor fails to activate Akt or promote cell survival in these cells. Association of Akt and PDK-1 with the membrane is also diminished in GqQ209L-expressing cardiomyocytes. Phosphatidylinositol 3,4,5-trisphosphate (PIP3), the primary regulator of Akt, increases significantly in GqWT-expressing cells but not in cardiomyocytes expressing GqQ209L. Levels of phosphatidylinositol 4,5-bisphosphate (PIP2), the immediate precursor of PIP3, are also markedly lower in GqQ209L-expressing compared to control cells. Expression of a GqQ209L mutant that has diminished capacity to activate phospholipase C does not decrease PIP2 or Akt or induce apoptosis. In transgenic mice with cardiac Gαq overexpression, heart failure and increased cardiomyocyte apoptosis develop during the peripartal period. Akt phosphorylation and PIP2 levels decrease concomitantly. Our findings suggest that an Akt-mediated cell survival pathway is compromised by the diminished availability of PIP2 elicited by pathol. levels of Gq activity.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 3 OF 13 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 2003509923 MEDLINE

DOCUMENT NUMBER: PubMed ID: 14585963

TITLE: Pyk2- and Src-dependent tyrosine phosphorylation of **PDK1** regulates focal adhesions.

AUTHOR: Taniyama Yoshihiro; Weber David S; Rocic Petra; Hilenski Lula; Akers Marjorie L; Park Jongsun; Hemmings Brian A; Alexander R Wayne; Griendling Kathy K

CORPORATE SOURCE: Department of Medicine, Division of Cardiology, Emory University School of Medicine, 1639 Pierce Drive, Atlanta, GA 30322, USA.

CONTRACT NUMBER: HL 38206 (NHLBI)
HL 58000 (NHLBI)
HL 60728 (NHLBI)

SOURCE: Molecular and cellular biology, (2003 Nov) 23 (22) 8019-29.
Journal code: 8109087. ISSN: 0270-7306.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200312

ENTRY DATE: Entered STN: 20031031
Last Updated on STN: 20031219
Entered Medline: 20031210

AB 3-**Phosphoinositide**-dependent protein kinase 1 (**PDK1**) is a signal integrator that activates the AGC superfamily of

serine/threonine kinases. **PDK1** is phosphorylated on tyrosine by oxidants, although its regulation by **agonists** that stimulate G-protein-coupled receptor signaling pathways and the physiological consequences of tyrosine phosphorylation in this setting have not been fully identified. We found that angiotensin II stimulates the tyrosine phosphorylation of **PDK1** in vascular smooth muscle in a calcium- and c-Src-dependent manner. The calcium-activated tyrosine kinase Pyk2 acts as a scaffold for Src-dependent phosphorylation of **PDK1** on Tyr9, which permits phosphorylation of Tyr373 and -376 by Src. This critical function of Pyk2 is further supported by the observation that Pyk2 and tyrosine-phosphorylated **PDK1** colocalize in focal adhesions after angiotensin II stimulation. Importantly, infection of smooth muscle cells with a Tyr9 mutant of **PDK1** inhibits angiotensin II-induced tyrosine phosphorylation of paxillin and focal adhesion formation. These observations identify a novel interaction between **PDK1** and Pyk2 that regulates the integrity of focal adhesions, which are major compartments for integrating signals for cell growth, apoptosis, and migration.

L29 ANSWER 4 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:62314 BIOSIS
DOCUMENT NUMBER: PREV200400062889
TITLE: Effects of chronic beta-adrenergic receptor stimulation in type 5 adenylyl cyclase-null mice.
AUTHOR(S): Okumura, Satoshi [Reprint Author]; Kawabe, Junichi [Reprint Author]; Yang, Guiping [Reprint Author]; Liu, Jing [Reprint Author]; Sadoshima, Junichi [Reprint Author]; Vatner, Stephen F. [Reprint Author]; Ishikawa, Yoshihiro [Reprint Author]
CORPORATE SOURCE: New Jersey Med Sch, Newark, NJ, USA
SOURCE: Circulation, (October 28 2003) Vol. 108, No. 17 Supplement, pp. IV-48. print.
Meeting Info.: American Heart Association Scientific Sessions 2003. Orlando, FL, USA. November 09-12, 2003. American Heart Association.
ISSN: 0009-7322 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Jan 2004
Last Updated on STN: 28 Jan 2004

L29 ANSWER 5 OF 13 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002204816 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11825911
TITLE: Protein kinase B is regulated in platelets by the collagen receptor glycoprotein VI.
AUTHOR: Barry Fiona A; Gibbins Jonathan M
CORPORATE SOURCE: School of Animal & Microbial Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, United Kingdom.
SOURCE: Journal of biological chemistry, (2002 Apr 12) 277 (15) 12874-8. Electronic Publication: 2002-02-01.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020409
Last Updated on STN: 20030105
Entered Medline: 20020516
AB **Phosphoinositide** 3-kinase (PI3K) is a critical component of the signaling pathways that control the activation of platelets. Here we have

examined the regulation of protein kinase B (PKB), a downstream effector of PI3K, by the platelet collagen receptor glycoprotein (GP) VI and thrombin receptors. Stimulation of platelets with collagen or convulxin (a selective GPVI **agonist**) resulted in PI3K-dependent, and aggregation independent, Ser(473) and Thr(308) phosphorylation of PKBalpha, which results in PKB activation. This was accompanied by translocation of PKB to cell membranes. The **phosphoinositide** -dependent kinase **PDK1** is known to phosphorylate PKBalpha on Thr(308), although the identity of the kinase responsible for Ser(473) phosphorylation is less clear. One candidate that has been implicated as being responsible for Ser(473) phosphorylation, either directly or indirectly, is the integrin-linked kinase (ILK). In this study we have examined the interactions of PKB, **PDK1**, and ILK in resting and stimulated platelets. We demonstrate that in platelets PKB is physically associated with **PDK1** and ILK. Furthermore, the association of **PDK1** and ILK increases upon platelet stimulation. It would therefore appear that formation of a tertiary complex between **PDK1** , ILK, and PKB may be necessary for phosphorylation of PKB. These observations indicate that PKB participates in cell signaling downstream of the platelet collagen receptor GPVI. The role of PKB in collagen- and thrombin-stimulated platelets remains to be determined.

L29 ANSWER 6 OF 13 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2002413471 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12167717
TITLE: Multiple **phosphoinositide** 3-kinase-dependent steps in activation of protein kinase B.
AUTHOR: Scheid Michael P; Marignani Paola A; Woodgett James R
CORPORATE SOURCE: Department of Experimental Therapeutics, University Health Network. Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.
SOURCE: Molecular and cellular biology, (2002 Sep) 22 (17) 6247-60. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020809
Last Updated on STN: 20020910
Entered Medline: 20020909

AB The protein kinase B (PKB)/Akt family of serine kinases is rapidly activated following **agonist**-induced stimulation of **phosphoinositide** 3-kinase (PI3K). To probe the molecular events important for the activation process, we employed two distinct models of posttranslational inducible activation and membrane recruitment. PKB induction requires phosphorylation of two critical residues, threonine 308 in the activation loop and serine 473 near the carboxyl terminus. Membrane localization of PKB was found to be a primary determinant of serine 473 phosphorylation. PI3K activity was equally important for promoting phosphorylation of serine 473, but this was separable from membrane localization. **PDK1** phosphorylation of threonine 308 was primarily dependent upon prior serine 473 phosphorylation and, to a lesser extent, localization to the plasma membrane. Mutation of serine 473 to alanine or aspartic acid modulated the degree of threonine 308 phosphorylation in both models, while a point mutation in the substrate-binding region of **PDK1** (L155E) rendered **PDK1** incapable of phosphorylating PKB. Together, these results suggest a mechanism in which 3' **phosphoinositide** lipid-dependent translocation of PKB to the plasma membrane promotes serine 473 phosphorylation, which is, in turn, necessary for **PDK1**-mediated phosphorylation of threonine 308 and, consequentially, full PKB activation.

L29 ANSWER 7 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2002679428 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12438101
TITLE: Cholinergic activation of glucose transport in bovine chromaffin cells involves calmodulin and protein kinase Czeta signaling.
AUTHOR: Serck-Hanssen Guldborg; Gronning Mona; Fladeby Cathrine; Skar Robert
CORPORATE SOURCE: Department of Physiology, University of Bergen, Bergen,Norway.. guldborg.serck-hanssen@fys.uib.no
SOURCE: Annals of the New York Academy of Sciences, (2002 Oct) 971 117-26.
Journal code: 7506858. ISSN: 0077-8923.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20021121
Last Updated on STN: 20021218
Entered Medline: 20021216

AB The aim of the present study was to delineate possible signaling pathways involved in acetylcholine (Ach)-induced glucose transport in chromaffin cells, a widely applied model system for sympathetic neurons. Acute Ach stimulation (10 min) enhanced the rate of glucose transport through activation of both nicotinic and muscarinic receptors. The calmodulin antagonist, W13, and the protein kinase C (PKC) inhibitor, staurosporine, each partially depressed Ach-induced glucose transport, with staurosporine exhibiting the stronger inhibitory effect. Pretreating the cells with phorbol 12-myristate 13-acetate (PMA) to downregulate PKC activity did not affect the nicotine-induced glucose transport, but completely attenuated that activated by muscarine, suggesting that Ach activation of transport involved both diacylglycerol-independent (PKCzeta) and diacylglycerol-dependent PKCs (PKCalpha/PKCepsilon). The PI 3-kinase inhibitor, wortmannin, diminished the Ach response, consistent with activation of the PKCs by the upstream PI 3-kinase-dependent **phosphoinositide**-dependent kinase, **PDK1**. Cholinergic activation strongly activated the ERK1/ERK2 cascade and p38 MAP kinase, but only p38 MAP kinase appeared to play a role, however minor, in nicotine-induced glucose uptake. The results are consistent with PKCs being more important than calmodulin in coupling cholinergic activation to glucose transport in chromaffin cells, but additional, yet unidentified, signaling pathways appear to be needed to obtain full activation of glucose transport in response to Ach.

L29 ANSWER 8 OF 13 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2001389026 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11373274
TITLE: Insulin-stimulated protein kinase B phosphorylation on Ser-473 is independent of its activity and occurs through a staurosporine-insensitive kinase.
AUTHOR: Hill M M; Andjelkovic M; Brazil D P; Ferrari S; Fabbro D; Hemmings B A
CORPORATE SOURCE: Friedrich Miescher Institute, Maulbeerstrasse 66, CH-4058 Basel, Switzerland.
SOURCE: Journal of biological chemistry, (2001 Jul 13) 276 (28) 25643-6. Electronic Publication: 2001-05-23.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010820
Last Updated on STN: 20030105
Entered Medline: 20010816

AB Full activation of protein kinase B (PKB, also called Akt) requires phosphorylation on two regulatory sites, Thr-308 in the activation loop and Ser-473 in the hydrophobic C-terminal regulatory domain (numbering for PKB alpha/Akt-1). Although 3'-**phosphoinositide**-dependent protein kinase 1 (**PDK1**) has now been identified as the Thr-308 kinase, the mechanism of the Ser-473 phosphorylation remains controversial. As a step to further characterize the Ser-473 kinase, we examined the effects of a range of protein kinase inhibitors on the activation and phosphorylation of PKB. We found that staurosporine, a broad-specificity kinase inhibitor and inducer of cell apoptosis, attenuated PKB activation exclusively through the inhibition of Thr-308 phosphorylation, with Ser-473 phosphorylation unaffected. The increase in Thr-308 phosphorylation because of overexpression of **PDK1** was also inhibited by staurosporine. We further show that staurosporine (CGP 39360) potently inhibited **PDK1** activity in vitro with an IC(50) of approximately 0.22 microm. These data indicate that **agonist**-induced phosphorylation of Ser-473 of PKB is independent of **PDK1** or PKB activity and occurs through a distinct Ser-473 kinase that is not inhibited by staurosporine. Moreover, our results suggest that inhibition of PKB signaling is involved in the proapoptotic action of staurosporine.

L29 ANSWER 9 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2001654900 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11707620
TITLE: Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms.
AUTHOR: Lang F; Cohen P
CORPORATE SOURCE: Department of Physiology, University of Tubingen, Germany.. florian.lang@uni-tuebingen.de
SOURCE: Science's STKE [electronic resource] : signal transduction knowledge environment, (2001 Nov 13) 2001 (108) RE17. Electronic Publication: 2001-11-13. Ref: 139
Journal code: 100964423. ISSN: 1525-8882.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011115
Last Updated on STN: 20020420
Entered Medline: 20020114

AB Serum- and glucocorticoid-induced protein kinase 1 (SGK1) was identified in 1993 as an immediate early gene whose mRNA levels increase dramatically within 30 minutes when cells are exposed to serum or glucocorticoids, or both. Subsequently, many other **agonists**, acting through a variety of signal transduction pathways, have been shown to induce SGK1 gene transcription in cells and tissues. SGK1 is a member of the "AGC" subfamily, which includes protein kinases A, G, and C, and its catalytic domain is most similar to protein kinase B (PKB). Like PKB, SGK1 is activated by phosphorylation in response to signals that stimulate phosphatidylinositol 3-kinase, and this is mediated by 3-**phosphoinositide**-dependent protein kinase 1 (**PDK1**) and another protein kinase that has yet to be identified. Thus, SGK1 is remarkable in being activated at both the transcriptional and posttranslational levels by a huge number of extracellular signals. In contrast, little is known about the transcriptional regulation of the two closely related isoforms SGK2 and SGK3, although they can be activated by

phosphorylation. The substrate specificity of SGK isoforms superficially resembles that of PKB in that serine and threonine residues lying in Arg-Xaa-Arg-Xaa-Xaa-Ser/Thr sequences (where Xaa is a variable amino acid) are phosphorylated. However, although they may have some substrates in common, evidence is emerging that SGK1 and PKB phosphorylate distinct proteins and have different functions in vivo. In particular, SGK1 plays an important role in activating certain potassium, sodium, and chloride channels, suggesting an involvement in the regulation of processes such as cell survival, neuronal excitability, and renal sodium excretion. Moreover, sustained high levels of SGK1 protein and activity may contribute to conditions such as hypertension and diabetic nephropathy. This raises the possibility that specific inhibitors of SGK1 may have therapeutic potential for the treatment of several diseases.

L29 ANSWER 10 OF 13 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:572690 SCISEARCH
THE GENUINE ARTICLE: 338LU
TITLE: **Phosphoinositide** 3-kinase-dependent phosphorylation of the dual adaptor for phosphotyrosine and 3-**phosphoinositides** by the Src family of tyrosine kinase
AUTHOR: Dowler S (Reprint); Montalvo L; Cantrell D; Morrice N; Alessi D R
CORPORATE SOURCE: Univ Dundee, Dept Biochem, MRC, Prot Phosphorylat Unit, MSI-WTB Complex, Dow St, Dundee DD1 5EH, Scotland (Reprint); Univ Dundee, Dept Biochem, MRC, Prot Phosphorylat Unit, Dundee DD1 5EH, Scotland; Fac Med, Dept Bioquim & Biol Mol, Madrid 28871, Spain; Imperial Canc Res Fund, Lymphocyte Activat Lab, London WC2A 3PX, England
COUNTRY OF AUTHOR: Scotland; Spain; England
SOURCE: BIOCHEMICAL JOURNAL, (15 JUL 2000) Vol. 349, Part 2, pp. 605-610.
ISSN: 0264-6021.
PUBLISHER: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 28
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We recently identified a novel adaptor protein, termed dual adaptor for phosphotyrosine and 3-**phosphoinositides** (DAPP1), that possesses a Src homology (SH2) domain and a pleckstrin homology (PH) domain. DAPP1 exhibits a high-affinity interaction with PtdIns(3,4,5)P-3 and PtdIns(3,4)P-2, which bind to the PW domain. In the present study we show that when DAPP1 is expressed in HEK-293 cells, the **agonists** insulin, insulin-like growth factor-1 and epidermal growth factor induce the phosphorylation of DAPP1 at Tyr(139). Treatment of cells with **phosphoinositide** 3-kinase (PI 3-kinase) inhibitors or expression of a dominant-negative PI 3-kinase prevent phosphorylation of DAPP1 at Tyr(130), and a PH-domain mutant of DAPP1, which does not interact with PtdIns(3,4,5)P-3 or PtdIns(3,4)P-2 is not phosphorylated at Tyr(139) following **agonist** stimulation of cells. Overexpression of a constitutively active form of PI 3-kinase induced the phosphorylation of DAPP1 in unstimulated cells. We demonstrated that Tyr(139) of DAPP1 is likely to be phosphorylated in vivo by a Src-family tyrosine kinase, since the specific Src-family inhibitor, PP2, but not an inactive variant of this drug, PP3, prevented the **agonist**-induced tyrosine phosphorylation of DAPP1. Src, Lyn and Lck tyrosine kinases phosphorylate DAPP1 at Tyr(139) in vitro at similar rates in the presence or absence of PtdIns(3,4,5)P-3, and overexpression of these kinases in HEK-293 cells

induces the phosphorylation of Tyr(139). These findings indicate that, following activation of PI 3-kinases, PtdIns(3,4,5)P-3 or PtdIns(3,4)P-2 bind to DAPP1, recruiting it to the plasma membrane where it becomes phosphorylated at Tyr(139) by a Src-family tyrosine kinase.

L29 ANSWER 11 OF 13 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1999208518 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10191262
TITLE: Activation of serum- and glucocorticoid-regulated protein kinase by **agonists** that activate phosphatidylinositide 3-kinase is mediated by 3-**phosphoinositide**-dependent protein kinase-1 (**PDK1**) and PDK2.
AUTHOR: Kobayashi T; Cohen P
CORPORATE SOURCE: MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, MSI/WTB Complex, Dow Street, Dundee DD1 5EH, Scotland, UK..
tkobayashi@bad.dundee.ac.uk
SOURCE: Biochemical journal, (1999 Apr 15) 339 (Pt 2) 319-28.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 20020420
Entered Medline: 19990623

AB The PtdIns(3,4,5)P3-dependent activation of protein kinase B (PKB) by 3-**phosphoinositide**-dependent protein kinases-1 and -2 (**PDK1** and PDK2 respectively) is a key event in mediating the effects of signals that activate PtdIns 3-kinase. The catalytic domain of serum- and glucocorticoid-regulated protein kinase (SGK) is 54% identical with that of PKB and, although lacking the PtdIns(3,4, 5)P3-binding pleckstrin-homology domain, SGK retains the residues that are phosphorylated by **PDK1** and PDK2, which are Thr256 and Ser422 in SGK. Here we show that **PDK1** activates SGK in vitro by phosphorylating Thr256. We also show that, in response to insulin-like growth factor-1 (IGF-1) or hydrogen peroxide, transfected SGK is activated in 293 cells via a PtdIns 3-kinase-dependent pathway that involves the phosphorylation of Thr256 and Ser422. The activation of SGK by **PDK1** in vitro is unaffected by PtdIns(3,4,5)P3, abolished by the mutation of Ser422 to Ala, and greatly potentiated by mutation of Ser422 to Asp (although this mutation does not activate SGK itself). Consistent with these findings, the Ser422Asp mutant of SGK is activated by phosphorylation (probably at Thr256) in unstimulated 293 cells, and activation is unaffected by inhibitors of PtdIns 3-kinase. Our results are consistent with a model in which activation of SGK by IGF-1 or hydrogen peroxide is initiated by a PtdIns(3,4, 5)P3-dependent activation of PDK2, which phosphorylates Ser422. This is followed by the PtdIns(3,4,5)P3-independent phosphorylation at Thr256 that activates SGK, and is catalysed by **PDK1**. Like PKB, SGK preferentially phosphorylates serine and threonine residues that lie in Arg-Xaa-Arg-Xaa-Xaa-Ser/Thr motifs, and SGK and PKB inactivate glycogen synthase kinase-3 similarly in vitro and in co-transfection experiments. These findings raise the possibility that some physiological roles ascribed to PKB on the basis of the overexpression of constitutively active PKB mutants might be mediated by SGK.

L29 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:645745 HCAPLUS
DOCUMENT NUMBER: 130:1665
TITLE: Regulation of protein kinase C ζ by PI 3-kinase

and PDK-1

AUTHOR(S): Chou, Margaret M.; Hou, Weimin; Johnson, Joanne; Graham, Lauren K.; Lee, Mark H.; Chen, Ching-Shih; Newton, Alexandra C.; Schaffhausen, Brian S.; Toker, Alex

CORPORATE SOURCE: Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104, USA

SOURCE: Current Biology (1998), 8(19), 1069-1077
CODEN: CUBLE2; ISSN: 0960-9822

PUBLISHER: Current Biology Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Protein kinase C ζ (PKCζ) is a member of the PKC family of enzymes and is involved in a wide range of physiol. processes including mitogenesis, protein synthesis, cell survival and transcriptional regulation. PKCζ has received considerable attention recently as a target of **phosphoinositide** 3-kinase (PI 3-kinase), although the mechanism of PKCζ activation is, as yet, unknown. Recent reports have also shown that the **phosphoinositide**-dependent protein kinase-1 (PDK-1), which binds with high affinity to the PI 3-kinase lipid product phosphatidylinositol-3,4,5-trisphosphate (Ptdlns-3,4,5-P3), phosphorylates and potently activates two other PI 3-kinase targets, the protein kinases Akt/PKB and p70S6K. We therefore investigated whether PDK-1 is the kinase that activates PKCζ. In vivo, PI 3-kinase is both necessary and sufficient to activate PKCζ. PDK-1 phosphorylates and activates PKCζ in vivo, and we have shown that this is due to phosphorylation of threonine 410 in the PKCζ activation loop. In vitro, PDK-1 phosphorylates and activates PKCζ in a Ptdlns-3,4,5-P3-enhanced manner. PKCζ and PDK-1 are associated in vivo, and membrane targeting of PKCζ renders it constitutively active in cells. Our results have identified PDK-1 as the kinase that phosphorylates and activates PKCζ in the PI 3-kinase signaling pathway. This phosphorylation and activation of PKCζ by PDK-1 is enhanced in the presence of Ptdlns-3,4-5-P3. Consistent with the notion that PKCs are enzymes that are regulated at the plasma membrane, a membrane-targeted PKCζ is constitutively active in the absence of **agonist** stimulation. The association between PKCζ and PDK-1 reveals extensive cross-talk between enzymes in the PI 3-kinase signaling pathway.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 13 OF 13 MEDLINE on STN

ACCESSION NUMBER: 1998111615 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9449962

TITLE: Cross-talk between phospholipase C and **phosphoinositide** 3-kinase signalling pathways.

AUTHOR: Batty I H; Hickinson D M; Downes C P

CORPORATE SOURCE: Department of Biochemistry, University of Dundee, U.K.

SOURCE: Biochemical Society transactions, (1997 Nov) 25 (4) 1132-7.
Ref: 33
Journal code: 7506897. ISSN: 0300-5127.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199804

ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 20000303
Entered Medline: 19980413

AB 1321N1 astrocytoma cells have proved a valuable model system in which to study interactions between two major PtdIns (4,5) P2-utilizing signaling pathways, since they possess receptor populations which elicit independent activation of PI 3-kinase and a G-protein-dependent PLC respectively. Activation of PLC down-regulates PI 3-kinase by at least two mechanisms involving inhibition of IRS-1-associated PI 3-kinase and acute activation of a PtdIns (3,4,5) P3 5-phosphatase. PKB, which is an important early PI 3-kinase-dependent component of insulin signalling pathways, is also down-regulated by PLC-coupled **agonists**. The activation of PKB by insulin appears to involve a novel PtdIns (3,4,5) P3-dependent protein kinase, which we have named **PDK1**. The molecular mechanisms underlying PtdIns (3,4,5) P3-stimulated phosphorylation and activation of PKB by **PDK1** are currently under investigation.

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(FILE 'HOME' ENTERED AT 16:25:51 ON 15 JUL 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 16:26:13 ON 15 JUL 2005

L1	1799	S	"PDK1"
L2	62622	S	PHOSPHOINOSITIDE##
L3	1051	S	L1 AND L2
L4	2934	S	"PIF" OR "PRK2"
L5	78	S	L3 AND L4
L6	24	DUP REM	L5 (54 DUPLICATES REMOVED)
L7	528	S	"SERINE 473"
L8	0	S	L6 AND L7
L9	35	S	L3 AND PKBALPHA
L10	19	DUP REM	L9 (16 DUPLICATES REMOVED)
L11	67	S	L3 AND "PDK2"
L12	24	DUP REM	L11 (43 DUPLICATES REMOVED)
L13	884	S	SER473 OR THR308
L14	4	S	L12 AND L13
L15	4	DUP REM	L14 (0 DUPLICATES REMOVED)
		E	LESSI D/AU
		E	ALESSI D/AU
L16	118	S	E3
		E	BALENDRAN A/AU
L17	45	S	E3-E5
		E	DEAK M/AU
L18	353	S	E3-E8
		E	CURRIE R/AU
L19	99	S	E3
		E	DOWNS P/AU
		E	DOWNES P/AU
L20	83	S	E3-E12
		E	CASAMAYOR A/AU
L21	110	S	E3
L22	764	S	L16 OR L17 OR L18 OR L19 OR L20 OR L21
L23	126	S	L3 AND L22
L24	15	S	L13 AND L23
L25	4	DUP REM	L24 (11 DUPLICATES REMOVED)
L26	16	S	L9 AND L22
L27	9	DUP REM	L26 (7 DUPLICATES REMOVED)
L28	37	S	L3 AND AGONIST?
L29	13	DUP REM	L28 (24 DUPLICATES REMOVED)

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	Issue Date	Pages	Document ID	Title
1	20050210	64	US 20050032185 A1	Enzyme
2	20050120	27	US 20050014682 A1	Cell-free assay for insulin signaling
3	20040805	33	US 20040152667 A1	4-Alkenylthiazoles comprising epoxide functionality, and methods of use thereof
4	20040115	176	US 20040009569 A1	Kinase crystal structures and materials and methods for kinase activation
5	20040108	134	US 20040005687 A1	Kinase crystal structures
6	20030731	90	US 20030143656 A1	Protein kinase regulation
7	20030731	22	US 20030143583 A1	Novel response element
8	20030612	63	US 20030108971 A1	Enzyme
9	20040511	61	US 6734001 B1	3-phosphoinositide-dependent protein kinase
10	20021029	23	US 6472515 B1	Response element

	Issue Date	Pages	Document ID	Title
1	20050210	64	US 20050032185 A1	Enzyme
2	20040115	176	US 20040009569 A1	Kinase crystal structures and materials and methods for kinase activation
3	20040108	134	US 20040005687 A1	Kinase crystal structures
4	20030612	63	US 20030108971 A1	Enzyme
5	20040511	61	US 6734001 B1	3-phosphoinositide-dependent protein kinase

	Issue Date	Pages	Document ID	Title
1	20050428	63	US 20050090541 A1	Indolinone derivatives and their use in treating disease-states such as cancer
2	20050120	27	US 20050014682 A1	Cell-free assay for insulin signaling
3	20040115	176	US 20040009569 A1	Kinase crystal structures and materials and methods for kinase activation
4	20040108	134	US 20040005687 A1	Kinase crystal structures
5	20030731	90	US 20030143656 A1	Protein kinase regulation
6	20020815	170	US 20020110811 A1	Variants of protein kinases

	Issue Date	Pages	Document ID	Title
1	20050428	63	US 20050090541 A1	Indolinone derivatives and their use in treating disease-states such as cancer
2	20050120	27	US 20050014682 A1	Cell-free assay for insulin signaling
3	20040115	176	US 20040009569 A1	Kinase crystal structures and materials and methods for kinase activation
4	20040108	134	US 20040005687 A1	Kinase crystal structures
5	20030731	90	US 20030143656 A1	Protein kinase regulation
6	20020815	170	US 20020110811 A1	Variants of protein kinases

	Issue Date	Pages	Document ID	Title
1	20050707	53	US 20050148640 A1	Aminofurazan compounds useful as protein kinase inhibitors
2	20050616	41	US 20050130977 A1	Inhibitors of akt activity
3	20050616	137	US 20050130954 A1	AKT protein kinase inhibitors
4	20050512	73	US 20050101594 A1	Compositions useful as inhibitors of protein kinases
5	20050428	63	US 20050090541 A1	Indolinone derivatives and their use in treating disease-states such as cancer
6	20050421	70	US 20050085436 A1	Method to treat conditions associated with insulin signalling dysregulation
7	20050224	45	US 20050044579 A1	Neurotransmitter signaling can regulate life span in C. elegans
8	20050217	43	US 20050037440 A1	Methods of identifying longevity modulators and therapeutic methods of use thereof
9	20050210	64	US 20050032185 A1	Enzyme
10	20050120	27	US 20050014682 A1	Cell-free assay for insulin signaling
11	20050106	114	US 20050003450 A1	Immunoaffinity isolation of modified peptides from complex mixtures
12	20041028	369	US 20040214817 A1	Diaminotriazoles useful as inhibitors of protein kinases
13	20040923	268	US 20040186118 A1	Chk-, Pdk- and Akt-inhibitory pyrimidines, their production and use as pharmaceutical agents

	Issue Date	Pages	Document ID	Title
14	20040923	47	US 20040186115 A1	Compositions useful as inhibitors of protein kinases
15	20040722	13	US 20040143117 A1	Inhibitors of akt activity
16	20040624	206	US 20040122016 A1	Compositions useful as inhibitors of rock and other protein kinases
17	20040624	15	US 20040122012 A1	Inhibitors of akt activity
18	20040617	17	US 20040116433 A1	Inhibitors of akt activity
19	20040617	16	US 20040116432 A1	Inhibitors of akt activity
20	20040603	154	US 20040106615 A1	Protein kinase inhibitors and uses thereof
21	20040603	24	US 20040106540 A1	Method of treating cancer
22	20040603	109	US 20040106148 A1	Polypeptides
23	20040527	121	US 20040102360 A1	Combination therapy
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39	20020416	32	US 6372467 B1	P54s6k and p85s6k genes, proteins, primers, probes, and detection methods
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	L #	Hits	Search Text
1	L1	177	"pdk1"
2	L2	2733	phosphoinositide$2
3	L3	69	l1 same l2
4	L4	98	"ser 473" or "thr 308"
5	L5	5	l3 same l4
6	L6	946	"PRK2" or "PIF"
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8	L8	1	"pkbalpha"
9	L9	1122	"pkb"
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13	L13	5411	ALESSI CURRIE BALENDRAN DOWNES CASAMAYOR
14	L15	6	l6 and l14
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